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Department of Zoology, University of Kerala
Kariavattom, Trivandrum, India 695581

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The samples were diluted 100–1000 fold and counted in a phase contrast microscope. Ten replicates were used to determine the strength of the OB in the suspension.

Bioassays

A Leaf disc bioassay with cauliflower leaves was used. Leaf discs were cut to a size of 33 mm diameter. GmNPV suspension (2×10^7 POB/ml) containing 0.01 per cent Tween 80 was applied at the rate of 10 μl on each side of the leaf disc and spread uniformly over the entire surface of the disc using a sterile glass rod with a rounded and polished end. Control discs were treated with distilled water containing 0.01% Tween 80 only. The larvae released on the discs were allowed to feed for 36 h. The larvae were then transferred to fresh untreated leaf bouquets contained in plastic jars. Mortality of larvae was recorded from 4–9 days after treatment. GmNPV extracted from the cadavers as per the methods described above was serially passaged through the larvae of *P. xylostella* 15 times. One hundred and fifty early third instar larvae were used for each passage and the virus obtained at the end of each passage was stored separately at −20 °C. The yield of OB/larva was also determined at the end of each passage.

The viral stock of each passage was semipurified as explained above, counted with a haemocytometer and diluted five times with the starting dose of 1167.2 OB/mm^2 and bioassayed against early third instar larvae of *P. xylostella* using the leaf disc bioassay technique. Thirty-three larvae were used per dose to determine the concentration and time-mortality responses.

Restriction endonuclease analysis

To study the genetic variation between the wild type and serially passaged GmNPV, the DNA of both were subjected to restriction endonuclease analysis using *Pst* 1 enzyme as per the methods described by Smith and Summers (1979). The molecular weight of the fragments was calculated on log molecular weight of co-migrating 1 kb ladder fragments and their migration distances were measured using Adobe Photoshop 5.0 software package. Curves were fitted and equations were generated using SPSS software to deduce the molecular weight of fragments.

Statistical analysis

The numbers (POB yield) were transformed to square root values before analysis of variance was performed using IRRISTAT ver. 3.1, Biometric Unit (IRRI, Philippines). LC_{50} and LT_{50} were calculated after transforming the concentrations to log doses and mortality to probits using SPSS ver 7.5 (USA).

RESULTS

Serial passage of GmNPV in *P. xylostella* resulted in increased virulence of the virus as evidenced by reduction in the LC_{50} of 15 times serially passaged GmNPV (4.47

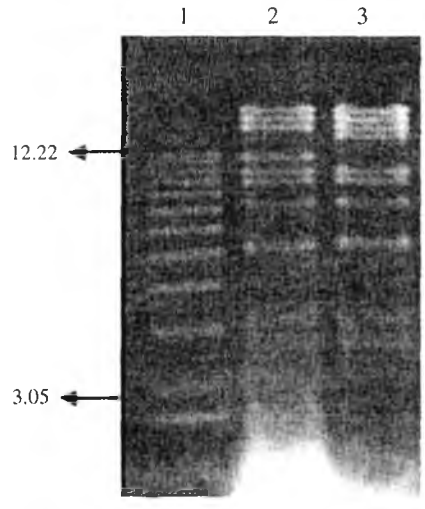

FIGURE 1. Restriction endonuclease analysis of GmNPV DNA with *Pst* I. Lane 1. Kb ladder; Lane 2. Control virus (wild-type GmNPV); Lane 3. Serially passed GmNPV in *P. xylostella*.

TABLE 1. Comparison of the molecular weights (Kbp) of DNA fragments of GmNPV generated by restriction endonuclease analysis—*Pst* I

Fragment Number	Molecular weights of DNA fragments (Kbp)	
	Wild type GmNPV	Serially passaged GmNPV
1	25.13	25.13
2	21.52	21.52
3	19.11	19.11
4	12.86	17.08
5	10.67	10.67
6	9.56	9.56
7	7.48	7.48
8	5.13	5.13
9	2.89	2.89
10	2.46	2.46
Total	116.82	121.04

OB/mm^2) when compared with wild-type GmNPV (54.84 OB/mm^2). There was 12.27 fold increase in virulence after 15 serial passages (Table 1).

LC_{50} of GmNPV was highest (85.09 OB/mm^2) at the end of third passage in *P. xylostella*. There was a significant reduction in LC_{50}, i.e. 18.04 and 8.02 OB/mm^2 after fourth and fifth passages respectively. LC_{50} declined in the subsequent passages except after 12 and 13th passages, which may be due to the variation in the host insect

TABLE 2. Effect of serial passage of GmNPV in *P. xylostella* larvae on the concentration-mortality responses of third instar *P. xylostella* larvae

No. of passages	No. observed	$\chi^2_{(n-2)}$*	Slope b ± S.E.	LC_{50} POB/mm2)	Fiducial limits	Yield (x 10^7 POB/larva) $	LT_{50} (hours)
1	197	0.282	0.446 ± 0.153	54.84	21.03–173.05	1.01 h	133.25
2	198	1.059	0.406 ± 0.150	57.06	19.99–208.72	1.30 b–f	135.42
3	193	2.831	0.508 ± 0.162	85.09	35.88–256.20	1.22 b–g	132.38
4	196	1.250	0.463 ± 0.148	18.04	6.46–46.71	1.19 d–g	119.48
5	198	4.270	0.331 ± 0.139	8.02	2.28–11.03	1.08 gh	108.65
6	196	0.612	0.433 ± 0.139	8.90	2.65–23.94	1.20 c–g	143.38
7	193	1.128	0.528 ± 0.141	4.72	1.56–10.97	1.16 e–h	121.34
8	198	3.383	0.372 ± 0.138	5.05	1.05–14.99	1.23 b–g	112.54
9	193	5.034	0.489 ± 0.140	6.41	2.00–16.11	1.14 fgh	127.79
10	197	4.704	0.466 ± 0.137	5.85	1.15–18.31	1.39 b	123.37
11	194	0.414	0.498 ± 0.140	6.74	2.26–16.20	1.20 b–e	135.71
12	193	0.351	0.489 ± 0.142	10.39	3.72–20.46	1.36 bcd	133.73
13	195	0.514	0.507 ± 0.142	11.31	4.00–28.24	1.39 bc	135.06
14	197	0.332	0.504 ± 0.138	5.40	1.78–12.83	1.73 a	144.99
15	194	2.562	0.537 ± 0.139	4.47	1.58–10.03	1.68 a	126.31

* All lines are significantly a good fit ($P < 0.05$).

$ Means followed by same letters are not different statistically ($P = 0.01$) by Duncan's multiple range test.

population tested. A sharp decline in LC_{50} after five serial passages in *P. xylostella* clearly indicates that GmNPV needs to be passaged through *P. xylostella* atleast five times for maximizing the virulence against the pest.

The yield of OB/larva increased with increase in the number of passages in *P. xylostella* and it was significantly high after 13 passages. The yield, however, increased by 67 per cent after 15 serial passages when compared to the yield from the wild-type virus. The serial passage of GmNPV in *P. xylostella* larvae had no effect on the LT_{50} which ranged from 108.65 to 144.99 h.

Restriction endonuclease (REN) profiles of control virus (wild-type) and passaged GmNPV DNA generated by *Pst* 1 enzyme showed similar banding patterns except for the absence of a 12.86 Kbp molecular size fragment in the passaged GmNPV DNA (Fig. 1). Instead, a 17.08 Kbp molecular size fragment was present in the passaged GmNPV DNA showing the deviation from the control GmNPV DNA. The molecular sizes of the control and passaged GmNPV DNA were 116.82 and 121.04 Kbp respectively (Table 2). This deviation in the molecular size of DNA confirms the genotypic variation in GmNPV following serial passages in *P. xylostella* larvae.

DISCUSSION

Failure of several management strategies to combat the notorious pest, *P. xylostella* (L.) has directed the attention in using baculoviruses with a broad host range. Isolation of a virulent variant of NPV in these heterologous infections is an attractive option. In the present study, there was a decline in LC_{50} when GmNPV was serially passaged through *P. xylostella* larvae 15 times. Decline in LC_{50} is in conformity with earlier observations by Kolodny-Hirsch and Van Beek (1997) that OBs from AcMNPV passaged serially 20 times through *P. xylostella* were approximately 15 times more virulent to second instar *P. xylostella* larvae than the wild type virus. They suggested that the basis for increased virulence of serially passaged virus is selection of genotypic variants characterized by OBs occluding a greater number of virions containing fewer nucleocapsids per envelope. OBs with greater number of virions would be expected to be more virulent than with fewer virions because there is more number of virus particles to initiate infection. It is suggested that virions containing fewer nucleocapsids could be more infectious, mainly because they would be more efficient in passing through the peritrophic membrane and binding to midgut cells (Tompkins *et al.*, 1988).

The yield of OBs/larva also increased following serial passages and the maximum yield of 1.73×10^7 OB/larva was observed after 13 passages. Vail (1973) proved that passage of AcNPV through *Trichoplusa ni, Spodoptera exigua* and *Estigmene acraea* resulted in highest mortality of *T. ni* larvae and yield of polyhedra.

There was a sharp decline in LC_{50} of GmNPV after three passages through *P. xylostella* larvae and then it steadily decreased in the subsequent passages. This might be due to the acquisition of host cell DNA sequences of baculoviruses upon serial passages through the host as suggested by Fraser *et al.* (1983). In the present study, this was proved by the difference in the banding patterns of GmNPV DNA before and

after passage through the host. Also, Kolodny-Hirsch and Van Beek (1997) showed minor differences in the REN fragment patterns after serial passage of AcNPV in *P. xylostella* larvae. DNA profiles of GmNPV generated using *Pst* I enzyme in the present study were identical with that obtained by Fraser *et al.* (1983) for GmNPV with the same enzyme. This further confirms the identity of GmNPV used in the present study and also the cross-infectivity of GmNPV to *P. xylostella*.

In general, NPVs adapt to the homologous or heterologous hosts upon sequential passages. Serial passages thus increase the virulence of a virus against a species that normally have low susceptibility to a given baculovirus. The positive effect of serial passage of NPV in homologous or heterologous hosts has been studied in different host-NPV interactions (Pavan *et al.*, 1981; Stairs *et al.*, 1981; Tompkins *et al.*, 1988). Although baculovirus activity may be improved through serial passages of wild-type isolates either on host or non-host insects, this method has not been adequately explored for many viruses including GmNPV. This study indicates the possibility of selection of a variant of GmNPV with increased virulence against the target pest, which is an attractive option for potentially increasing the utility of GmNPV as a viral insecticide.

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Diversity and trophic categorization of aquatic insects of Courtallam hills of Western Ghats

S. Anbalagan*[1], B. Kaleeswaran[2] and C. Balasubramanian[2]

[1]*Centre for Research in Aquatic Entomology, Department of Zoology, Madura College, Madurai 625011, India*
Email: anbalaganmdu@hotmail.com
[2]*Department of Zoology, Thiagarajar College, Madurai 625009, India*

ABSTRACT: Diversity and trophic categorization of aquatic insects of five falls stream of Courtallam hills of Western Ghats were investigated. A total of 2570 individuals of aquatic insects belonging to 23 genera, 19 families and 9 orders were collected in 4 stations in one second order streams for 6 months in Courtallam hills of Western Ghats. Diversity values of aquatic insects of five falls stream of Courtallam hills were calculated. Shannon index (H) and Simpson index (S) is highest in altitude 445 msl and lowest in altitude 310 msl. In five falls stream, there is a preponderance of genera of Ephemeroptera followed by Trichoptera and Plecoptera. The life cycle patterns of all the investigated seven species of mayflies are basically multivoltine with asynchronous, overlapping generations and continuous emergence. Trophic studies reveal that all sites in five falls stream show collector dominance followed by grazers and predators. Shredders were conspicuous by their absence. In terms of abundance of trophic categories, collectors were most abundant followed by grazers and predators.

KEYWORDS: Diversity, trophic categorization, aquatic insects, Western Ghats

INTRODUCTION

As a part of Post-Rio efforts to inventorize and monitor aquatic biodiversity, the study of diversity of aquatic insects of hill streams has gained lot of momentum in the past decade in our country. Aquatic insects form a dominant component of zoobenthos in montane lotic ecosystems (Dudgeon, 1999). They form vital links in fish food chain and are sensitive bioindicators of aquatic pollution. Some taxa are sensitive to global climatic change and are indicators of habitat health (Vinson and Hawkins, 1998). Western Ghats is one of the twenty five biodiversity hot spots in the world with over two third of its species of its amphibians and about a third of its angiosperms unique to that region. Endemism is hardly studied in insects except butterflies and a tenth

*Corresponding author

of that are endemic to the Western Ghats (Myers *et al.*, 2000). There have been a few valuable contributions on community-level investigations highlighting variation at local to regional scales, variation from headwater to the tail end in a river basin, as well as diversity studies restricted to a hill or a valley (Burton and Sivaramakrishnan, 1993). The objectives of the present investigation include the study of diversity and trophic categorizations of aquatic insects of Courtallam hills of Western Ghats.

MATERIALS AND METHODS

Study area

Courtallam is situated in the Western Ghats lying in the northern half of Tirunelveli District, Tamilnadu between 8° 50′ and 9° 0′ northern latitudes and 77° 10′ and 77° 20′ eastern longitudes. Courtallam is a hilly region whose height varies from 150 meters to 1500 meters with narrow valleys endowed with sleep slopes. Four stations were selected from one of these tributaries, of which three are located above the five falls and one below the five falls. During the monsoon, rains may be torrential, at time gentle and it may last for several days. The total amount rainfalls of Courtallam generally ranges from 175–210 cms per year. Rainfall is not evenly distributed throughout the year. Maximum rainfall is from September and October to December, during Southwest monsoon and Northeast monsoon respectively. The study was conducted from July 2002 to December 2002. The basic habitat consists of a series of rocky edges overlain with large boulders and rubble. The substratum is pebble and gravel integrated with coarse sand in quieter water at the edges. The stream averages 15 m wide with a maximum depth of 2 m. Along the banks of the stream are thick stands of trees and shrubs whose leaves are the stream's principal source of organic detritus. Among the taller plants are *Pongamia* sp., *Artocarpus* and *Terminalia* sp. Because of the leaf canopy over the stream formed by branched and leaning trees there is feeble exposure to direct sunlight even in mid day. So fluctuation in day and night temperature is minimized.

Sampling methods

The aquatic insects were quantitatively sampled using a 1 m wide Kick-net (Burton and Sivaramakrishnan, 1993) with mesh size of about 1mm. Riffle/run areas of stream were selected for sampling, since Kick-net sampling works best in these areas. One person held the Kick-net while the other person systematically sampled the 1 m^2 area. Every large boulder or cobble in this area was picked up if it could be lifted and organisms were vigorously washed by hand into the net. The specimens were then carefully picked form the net surface and were preserved immediately in 70% ethyl alcohol. These samples were transported to the laboratory for further processing. All specimens from each of the 4 sites in one stream were sorted and identified with the help of field guide (Sivaramakrishnan *et al.*, 1998). Further identification of the aquatic insect taxa were confirmed by specialists working in respective groups. Physico-

chemical parameters (atmospheric and water temperature, current velocity and pH) were recorded during collection time.

Mouth part morphology and gut content analysis of the more common taxa were used to categorize the functional feeding groups based on Cummins and Wilzbach (1985).

Primarily two-diversity indices *viz.*, Shannon-Weiner index (alpha diversity) and Simpson index (alpha diversity) were worked out.

The Shannon index of diversity (H) was calculated using the following formula,

$$\mathbf{H}' = \sum_{i}^{s} = 1(p_i/N)\log_2(p_i/N).$$

Where, N is the total number of individuals, pi is the number of individuals in the ith species and the information content or diversity is expressed as a number of bits (Ludwig and Reynolds, 1988).

Simpson index of diversity was calculated using the equation,

$$D = \sum_{i=1}^{s} P_i^2.$$

Where, P_i is the proportion of the ith species (Ludwig and Reynolds, 1988).

Developmental stages of may fly nymphs were classified following, the plan of Clifford (1969). Nymphs were counted. Each nymph was grouped into one of the four arbitrarily chosen developmental stages on the basis of the appearance and development of the mesothoracic wing pads. Stage I nymphs lacked wing pads, stage II nymphs had wing pads but their lengths were shorter than the distance separating the two wing pads; the wing pad length of stage III nymph was greater than the distance separating the two wing pads. Stage IV nymphs had darkened wing pads. Each stage represents several instars with the exception of stage IV, which is the last nymphal instar, the tan wing pads indicating impending emergence.

RESULTS AND DISCUSSION

A total of 2570 individuals of aquatic insects belonging to 23 genera, 19 families and 9 orders were collected by semi-quantitative kick-net sampling (3 in each) and limited opportunistic collections were made in four stations in one second order streams for 6 months (from July, 2002 to December, 2002) in Courtallam hills of Western Ghats (Table 1). Shannon index is 2.493 in altitude, 445 msl and 1.883 in altitude, 310 msl. Simpson's index is 6.620 in altitude, 445 msl and 4.077 in altitude, 310 msl. It is interesting to compare the aquatic insects assemblage pattern of the five falls stream with other hill streams in South India. In fact, studies of broad scale pattern of aquatic insect composition in all continents have revealed the remarkable worldwide similarity among stream insect assemblages. Indeed, many of the same families and genera of aquatic insects are found worldwide (Vinson and Hawkins, 1998). At the macroscale level, when a comparison is made with similar second order streams in temperate

TABLE 1. Taxonomic inventory and trophic categorization of aquatic insects of five falls stream of Courtallam

Order	Family	Genus	Trophic category
Ephemeroptera	Heptageniidae	*Epeorus* sp.	Collector
	Heptageniidae	*Cinygmina* sp.	Collector
	Heptageniidae	*Thalerosphyrus* sp.	Grazer
	Leptophlebiidae	*Indialis* sp.	Collector
	Leptophlebiidae	*Isca* sp.	Collector
	Ephemerellidae	*Teloganodes* sp.	Collector
	Baetidae	*Baetis* sp.	Grazer
Trichoptera	Hydropsychidae	*Potamyia* sp.	Collector
	Hydropsychidae	*Hydropsyche* sp.	Collector
	Polycentropodidae	*Polycentropus* sp.	Collector
	Rhycophilidae	*Atoposyche* sp.	Predator
	Philopotamidae	*Wormaldia* sp.	Collector
Plecoptera	Perlidae	*Neoperla* sp.	Predator
Megaloptera	Corydalidae	*Corydaleus* sp.	Predator
Odonata	Gomphydae	Progomphus sp.	Predator
	Coenagryonidae	*Coennagryon* sp.	Predator
	Libellulidae	*Pantala* sp.	Predator
Coleoptera	Psephenida	*Psephenoides* sp.	Grazer
Diptera	Simulidae	*Simulium* sp.	Collector
Hemiptera	Naucoridae	*Naucoris* sp.	Predator
	Gerridae	*Gerris* sp.	Predator
Orthoptera	Gryllotalpidae	*Grylloptalpa* sp.	Predator
	Tetrigidae	*Tridatylus* sp.	Collector
9 Orders	19 Families	23 genera	

ecosystems, there is less diversity or at least comparable diversity with temperate counterparts. Yet another factor while making such a temperate tropical comparison depend on the group of insects compared. For instance riffle beetles are more diverse in the tropics (Brown, 1981) while others, like Plecoptera, area more diverse in temperate streams (Zwick, 1986). In the present investigation, 7 out of 23 genera of aquatic insects collected belong to Ephemeroptera.

At the microscale level, factors like substrate, habitat type, light, food, water, temperature, stream size and flow pattern exert major influences in structuring benthic insect diversity. Physico-chemical parameters (atmospheric and water temperature, current velocity and pH) recorded at four stations of five falls stream is presented in Table 2. In present investigation 13 genera of Ephemeroptera, Plecoptera and Trichoptera were recorded. South Indian hill streams, in general, usually have

TABLE 2. Physicochemical features, water quality parameters and diversity index for individual sites

Site	Altitude (msl)	pH	Water temperature	Current speed (sec m^{-1})	Substrate	Diversity index	
						Shannon –Wiener index	Simpson index
1	445	7.2	22	0.4 m/sec	Bed rock with pebbles and 20–40 diameter sandy	2.493	6.620
2	425	7.3	22	0.5 m/sec	Bed rock with boulders and pebbles	2.330	5.992
3	380	7.0	22	0.2 m/sec	Bed rock, large boulders and pebbles	2.050	5.081
4	310	8.2	23	0.2 m/sec	Rock and sand mixed with silt	1.883	4.077

preponderance of Trichoptera followed by ephemeropteran and plecopteran genera. This pattern is exhibited by most of the Western Ghats streams, whereas in five falls there is a preponderance of the genera of Ephemeroptera followed by Trichoptera and Plecoptera. The list of trophic categories of aquatic insects collected in sampling sites for six months in four sites in five falls stream is presented in Table 1. All sites in five falls stream show collector (60%) dominance followed by grazers (20%) and predators (20%). Shredders were conspicuous by their absence. In terms of abundance of trophic categories, collectors were most abundant, followed by grazers and predators. The functional feeding group analysis in five falls streams showed considerable similarity with studies on the Western Ghats stream in peninsular India (Burton and Sivaramakrishnan, 1993). The preponderance of collectors in tropical streams may be due to the fact that leaves are decomposed to detrital particles by the microbial community in matter of days leaving little for shredder to feed as pointed out by Burton and Sivaramakrishnan (1993).

Life cycle patterns of seven species namely *Epeorus petersi*, *Cinygmina kumbakkariensis*, *Thalerosphyrus flowersi*, *Indialis* sp., *Isca* sp., *Teloganodes* sp., and *Baetis* sp. In five falls stream indicate them to be basically multivoltine with asynchronous, overlapping generations and continuous emergence. It is of interest to compare the investigations of Sivaramakrishnan and Job (1981) on the life cycle patterns of *Petersula Courtallensis* and *Notophlebia jobi* in Courtallam with the present investigated species. However, the local influence of the two monsoons is especially felt in abruptly terminating the cycles at the end of the monsoons due to the dwindling discharge from the headwaters. Similar life cycle patterns were recorded in heptagenid mayflies of Kumbakkari stream of the Western Ghats by Venkataraman (1984).

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Article No. ent.29303

In support of synonymisation of *Cassida nilgiriensis* (Borowiec and Takizawa) with *Cassida circumdata* (Hbst.) and of *Aspidimorpha lobata* Boh. with *A. sanctaecrucis*, F. (Coleoptera: Chrysomelidae: Cassidinae)

T. Kalaichelvan[1] and K. K. Verma*[2]

[1]*Maitri Baag Zoo, Bhilai Steel Plant, Bhilai, Chhattisgarh 400006, India*
[2]*HIG-1/327, Housing Board Colony, Borsi, Durg, Chhattisgarh 491001, India*
Email: kkverma@kkverma.com

ABSTRACT: Borowiec (1999) synonynmised *C. nilgiriensis* with *Cassida circumdata* on the basis of observations of Ghate and his school in Pune. Additional observations supporting this synonymisation, including results of breeding experiments, study of structure of aedeagus, food plants, feeding pattern and developmental stages are presented in this communication.

Synonymisation of *Aspidimorpha lobata* Boh, with *A. sanctaecrucis*. F. suggested by Maulik, 1919 has found support in our study, including breeding experiments, aedeagal structure, choice of food plants and feeding pattern.

KEYWORDS: Synonymisation, *Cassida circumdata*, *C. nilgiriensis*, *Aspidimorpha sanctaecrucis*, *A. lobata*, feeding pattern, aedeagus, breeding experiments

INTRODUCTION

Cassida circumdata Hbst., is a very common tortoise beetle in South Asia, with its range extending from the Indian subcontinent to Taiwan and Philippines. Another and similar species *Cassida nilgiriensis* was described from within this range by Borowiec and Takizawa (1991). The latter species differs from the former in having completely dark discs of the elytra and in the presence of two pairs of dark spotches in the elytral explanate margins, one pair humeral and one pair posterolateral. H. V. Ghate of Pune (pers. communication) found individuals, having features of both the species, developing from similar larvae and pupae. Ghate and his coworkers arranged

*Corresponding author

experimental crosses between *C. nilgiriensis* male and *C. circumdata* female and vice versa. In the progeny, resulting from these crosses there were individuals with characteristics of both the 'species'. In view of these observations of the Pune school Borowiec (1999), in his World Catalogue of Cassidines, synonymised *C. nilgiriensis* with *C. circumdata*. Being unaware of the results of the Pune school until recently, we also made observations which support this synonymisation.

Aspidimorpha lobata Boh. was synonymised with *A. sanctaecrucis*, F. by Maulik in 1919, and this was followed by Kimoto *et al.* (1995). But in recent publications on Cassidinae (Borowiec, 1999; Swietojanska, 2001) the two have been treated as separate species.

A. sanctaecrucis populations in Drug–Bhilai (Central India) include two types of variants among adults: (i) Those having dark brown elytra, lighter around the scutellum, and getting darker towards the edges. In this variant the spotches on the explanate margin of the elytra are as dark as the outer parts of the discs of the elytra. (ii) Those with reddish yellow colour of the elytral disc with a golden shine, which disappears on drying and storage of the specimen. Borowiec (pers. communication, dated 31st Dec. 2002) on seeing the VCD of live specimens. prepared by us, identified the specimen of the type (ii) as *A. lobata*, while specimens of the type (i) were labelled in the recording as *A. sanctaecrucis*. It is these two categories of insects which have been studied by us.

We are presenting our observations here to strengthen these cases of synonymisation.

MATERIAL AND METHODS

Breeding experiments, described in this communication, were performed in culture jars with mouth covered with a piece of thin cloth, held in place with a rubber band. In the jars the insects were kept with leaves of the food plants (*Ipomoea aquatica, I. batatas, I. Palmata* and *I. violacea*) in case of *C. nilgiriencis/C. circumdata*, and *I. aquatica* and *I. fistulosa* in case of *A. lobata* and *A. sanctaecrucis*. The leaves were regularly changed to make fresh food available to the insects. Observations on feeding pattern have been made in the field; they have been repeated in cultures also. Aedeagi of the four 'species' have been dissected and examined. Insects have been collected from parks of Bhilai Township.

OBSERVATIONS

Breeding experiments

On 18.02.99 a female of *C. nilgiriensis* was collected from a leaf of *Ipomoea aquatica* in Sector-8 Park, Bhilai. It was kept in a culture jar, and was provided with fresh leaves of *I. aquatica*. It was presumably a mated female, and after a few days it started oviposition. The oothecae were very similar to those of *C. circumdata*. As soon as adults in the progeny ecloded, they were removed from the culture and were examined for their body colouration. After a few days the female laid another batch of eggs.

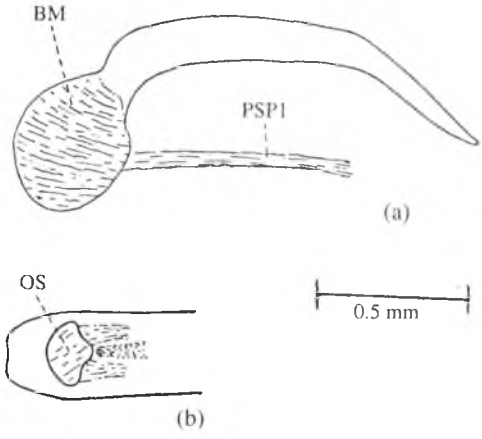

FIGURE 1. Aedeagus of *Cassida circumdata* (typical). (a) in lateral view. (b) apical part in dorsal view (the organ is slightly tilted to the left to the insect). N.B.: The '*C. nilgiriensis*' morph has a very similar aedeagus, BM = basal muscular bulb, OS = ostium, PSP1 = protractor of the tegmen or the first spiculum.

Again freshly ecloded adults were removed. In total among the progeny we found 12 *C. circumdata* (with typical body colour), 7 *C. circumdata* (with faded colour) and 19 *C. nilgiriencis*.

We kept the adults, obtained from the above experiment in 3 culture jars immediately after eclosion with different combinations of the two sexes from *C. circumdata* (typical) *C. circumdata* (faded) and *C. nilgiriencis*. We again got among the resulting progeny individuals with all the three types of body colouration.

One field collected female of *A. sanctaecrucis* was kept in a culture jar, with leaves of *Ipomoea fistulosa*. She laid oothecae from which larvae hatched out, changed into pupae and eventually adults emerged. The freshly emerging adults were greenish white; subsequently, after 3 to 4 days, their colour darkened, and after 12 to 14 days, 18 adults among the progeny showed the colouration of *A. sanctaecrucis* and 13 of *A. lobata*.

Developmental stages

It was observed that very similar largvae and pupae, agreeing with the description of George and Venkataraman (1987), develop into adults of *C. circumdata* and *C. nilgiriensis*.

Aedeagus

Aedeagi of typical *C. circumdata* and of *C. nilgiriensis* were dissected out and examined. The long slender aedeagus, with a basal muscular bulb and spatula like tip [Fig. 1(a) and (b)], showed no difference between the two 'species'.

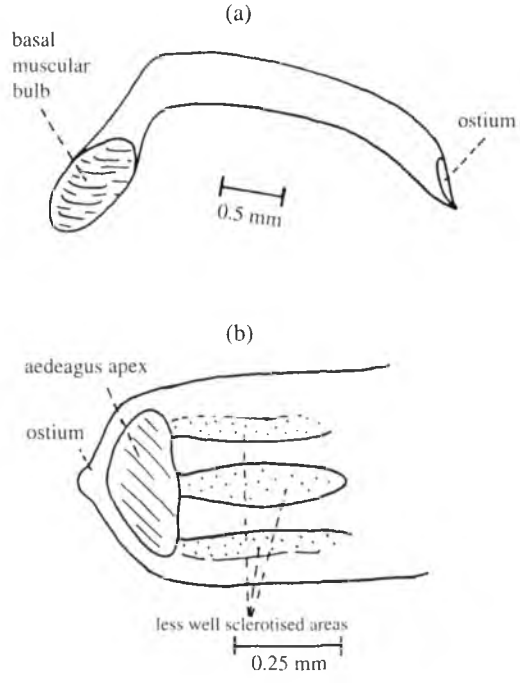

FIGURE 2. Aedeagus of *Aspidimorpha lobata* (a) in lateral view (b) apical part in dorsal view.

Aedeagi of *A. lobata* and of *A. sanctaecrucis* have been examined and found to be identical. Fig. 2 (a and b) are for *A. lobata*, and may as well be taken for *A. sanctaecrucis*.

Feeding pattern

Feeding pattern on leaves of *Ipomoea violacea* is very similar for *C. circumdata* and *C. nilgiriensis* [Fig. 3(a) and (b)].

The food plants, namely *Ipomoea aquatica, I. batatas, I. fistulosa, I. palmata, I. violacea, Merremia emerginata* and *M. tridentate*. are also common for the two 'species'.

Both *A. lobata* and *A. sanctaecrucis* adults have been collected from the same host plants in Durg–Bhilai; the plants included *Ipomoea aquatica* and *I. fistulosa*. The feeding pattern [see Figs. 4(a) and (b)] of the two type of individuals are completely similar.

Pecten on tarsal claws

The pecten is similar and equally well developed in adults of *A. sanctaecrucis* and *A. lobata* (Fig. 5a and b). It may be pointed out here that as per Swietojanska (2001) the pecten on tarsal claws of the two 'species' are different.

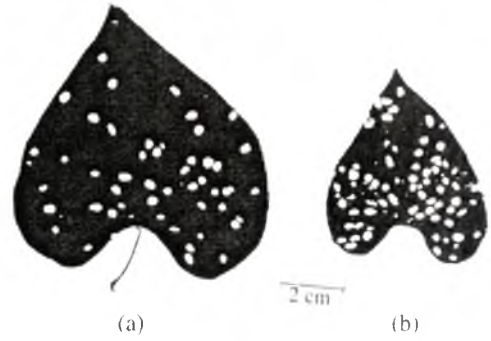

FIGURE 3. Feeding pattern on leaf of *Ipomoea violacea* (a) of adult of *Cassida circumdata* (typical) (b) of adult of *C. nilgiriensis*.

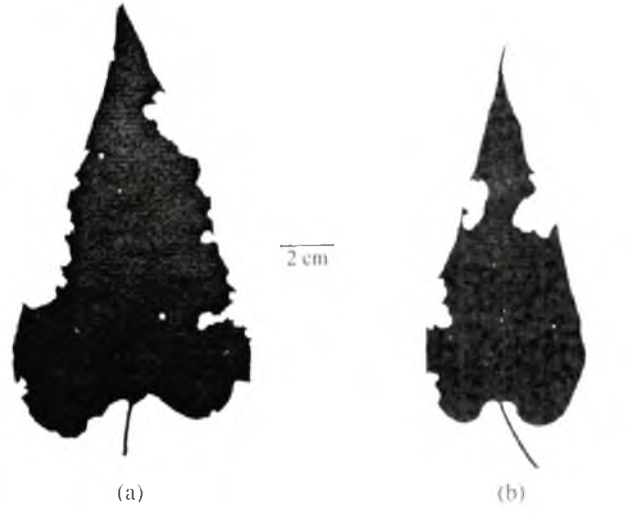

FIGURE 4. Feeding pattern of a leaf of *Ipomoea fistulosa* (a) by an adult of *Aspidimorpha sanctaecrucis* (b) by an adult of *A. lobata*.

DISCUSSION

Observation of the Ghate school of Pune and those presented in this paper provide adequate basis for synonymisation of *Cassida nilgiriensis* (Borowiec and Takizawa) with *Cassida circumdata* (Hbst.).

Our observations also clearly support the synonymisation of *Aspidimorpha sanctaecrucis* F. and *Aspidimorpha lobata* Boh.. Of the two species names *Aspidimorpha* (= *Aspidomorpha sanctaecrucis* is the older. Hence *A. lobata* may be taken as junior synonym of *A. sanctaecrucis*. Similarly the name *Cassida circumdata*, being the older of

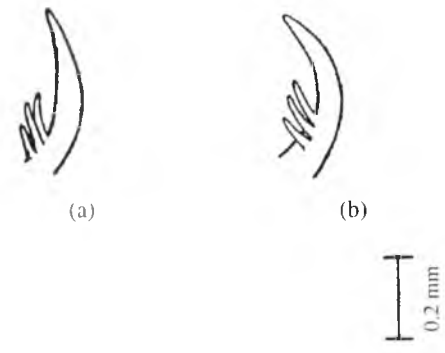

FIGURE 5. A metathoracic leg tarsal claw with pecten (a) of *Aspidimorpha sanctaecrucis* (b) of *Aspidimorpha lobata*.

the two names *C. circumdata* and *C. nilgiriensis*, be taken as the name for the species and *C. nilgiriensis* as a junior synonym.

In these studies we have taken into account observations on breeding, aedeagal structure and feeding pattern. Significance of these supports in synonymisation of species has been pointed out in Kalaichelvan *et al.* (in press) and Verma and Kalaichelvan (in press).

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Studies on morphometrics and biology of citrus butterfly *Papilio demoleus* Linnaeus (Lepidoptera: Papilionidae) on acid lime, *Citrus aurantifolia* Swingle

A. Rama Krishna Rao*[1], T. Murali Krishna[2], S. V. R. K. Rao[1], B. Chenchu Reddy[2], K. Hari Babu[1] and K. Gopal[1]

[1]*All India Co-ordinated Research Project on Tropical Fruits (Citrus), Tirupati 517502, Andhra Pradesh, India*
[2]*Regional Agricultural Research Station, Tirupati 517502, Andhra Pradesh, India*

ABSTRACT: The biology of citrus butterfly was studied under laboratory conditions on balaji acid lime. Adult female laid eggs singly or in groups of two to five on the under surface of tender leaves and also on tender twigs. Oviposition usually took place during the night. The average number of eggs laid by a gravid female moth was 119. Average incubation period was 3.65 days. Five larval instars were recorded. The first, second, third, fourth and fifth instar larvae lasted for 2.97, 3.27, 3.98, 3.37 and 3.99 days, respectively, on the average. The prepupal period lasted for 1.00 to 1.05 days while the pupal period lasted for 8.50 to 9.50 days. The total life cycle of citrus butterfly on balaji acid lime lasted for 29.90 to 32.61 days with an average of 31.36 days. The average longevity of male and female was 3.87 and 6.86 days, respectively.

KEYWORDS: Citrus butterfly, *Papilio demoleus*, biology

INTRODUCTION

Citrus is one of the most important commercial crop of the world and cultivated in the tropical and subtropical regions. Citrus industry is the third largest fruit industry in the world occupying six per cent of the total area under various fruits. In Andhra Pradesh acid lime occupies an area of 40,672 ha with an annual production of 2.27 lakh tonnes of fruits (Directorate of Economics and Statistics, A.P. 2001–2002). Balaji acid lime is a clonal selection which exhibits high resistance towards citrus canker (*Xanthomonas citri*) and also high yielder. Pest problem is one of the major constraints in the production of acid limes. According to Bhutani (1979) citrus trees in India are attacked by more than 250 insect pests alone at all stages of growth right from

*Corresponding author

budlings and seedlings in nurseries. Out of these, 165 species are important in India causing an estimated loss of 30 per cent in yield (Pruthi and Mani, 1945). Among the various insect pests that attack citrus, the citrus butterfly, *Papilio demoleus* Linnaeus is a serious and a regular pest in nurseries, young flush and also on young seedlings. The caterpillars feed voraciously and cause extensive damage to nurseries and young seedlings leaving behind midribs only. Severe infestation resulted in entire defoliation of the tree (Bhutani and Jotwani, 1975) and leads to retardation plant growth and decreases fruit yield (Pruthi, 1969). Information on the morphometrics and biology of citrus butterfly on balaji acid lime will be useful to evolve effective management strategy, against this pest. It was in this context that the present study was undertaken.

MATERIALS AND METHODS

The biology of citrus butterfly, *Papilio demoleus* L. on balaji acid lime was studied during October and November of 2002 in the insectary by collecting fifth instar larvae regularly from balaji acid lime nurseries of A.I.C.R.P. on Tropical Fruits (Citrus), Tirupati. The larvae were reared in glass jars by providing fresh balaji acid lime leaves daily. The male and female adults obtained were released in cages for egg laying. The adult moths were provided with 10 per cent honey solution in cotton swabs that were hanged down in the cages throughout the period of egg laying in which tender balaji acid lime twigs were placed in conical flasks containing water. The twigs were changed after every 24 hours. After egg laying, the eggs were transferred to petridishes containing fresh tender balaji acid lime leaves in petridishes at room temperature. A moistened filter paper was kept in each petriplate to prevent the drying of leaves. The caterpillars were maintained in petridishes upto third instar. Later on they were transferred and reared in separate glass troughs and allowed them to complete first generation. Observations were recorded daily on the development of colour, size and the duration of different instars of larvae. The body length, width and width of head capsule of first instar (ten larvae) and eggs were measured by using ocular micrometer while second instar to fifth instar, pre-pupal and pupal morphometric data were recorded by using a standard graphic paper method. The duration of prepupal, pupal stages and changes in colour were also recorded and analyzed statistically (Sukhatme and Amble, 1985). Fecundity studies were made by releasing individual pairs of freshly emerged five paired adults and they were kept separately in cages containing fresh tender twigs of balaji acid lime placed in Hoagland solution. Observations regarding pre-mating, mating, pre-oviposition, ovi-position and post-oviposition periods for females and adult longevity, body length, width, wing expanse and sex-ratio for both males and females were also recorded.

RESULTS AND DISCUSSION

The duration of different stages are presented in Table 1 and the morphometric data in Table 2.

TABLE 1. Duration of different stages in the life cycle of *Papilio demoleus* L. on acid lime

Particulars/stage	Duration (in days/hours)			SD	SEM
	Minimum	Maximum	Mean		
Pre-maturing period	11.00 (hours)	13.50 (hours)	12.10 (hours)	1.075	0.340
Mating period	1.60 (hours)	2.10 (hours)	1.89 (hours)	0.145	0.046
Pre-oviposition period	1.45	1.65	1.56	0.072	0.023
Oviposition period	4.10	4.45	4.32	0.139	0.044
Post oviposition period	4.00 (hours)	4.75 (hours)	4.09 (hours)	0.548	0.173
Incubation period	3.50	3.75	3.65	0.082	0.026
Larval periods					
a. First instar	2.80	3.15	2.97	0.133	0.042
b. Second instar	3.00	3.50	3.27	0.201	0.064
c. Third instar	3.90	4.10	3.98	0.075	0.024
d. Fourth instar	3.25	3.46	3.37	0.103	0.033
e. Fifth instar	3.95	4.10	3.99	0.043	0.014
Total larval period	16.90	18.31	17.64	0.653	0.206
Pre-pupae	1.00	1.05	1.20	0.021	0.007
Pupae	8.50	9.50	9.14	0.393	0.124
Longevity of adults		(with 10% sugar solution)			
a. Male	3.75	4.00	3.87	0.100	0.032
b. Female	6.75	6.95	6.86	0.077	0.024
Total life cycle (egg to adult)	29.9	32.61	31.36	1.348	0.426

Egg

Adult females lay eggs singly or in groups of two to five on the under surface of tender leaves and also on tender twigs by curling its abdomen. The freshly laid eggs measured 0.91 mm to 1.06 mm with an average of 0.99 mm and, are smooth, spherical, creamy yellow in colour and they turned to grayish with brown streaks all over the chorion before hatching. These descriptions agree with the reports of Atwal (1964), Ganguli and Ghosh (1967); Maheswarababu (1988). The average diameter of the egg was found to be 0.99 mm. Atwal (1964); Ganguli and Ghosh (1967); Resham *et al.* (1986); Maheswarababu (1988) also observed more or less similar trends in size of the egg. Incubation period ranged from 3.50 to 3.75 days which is slight variation with the findings of Maheswarababu (1988) who reported that the average incubation period lasted 2.96 days. Sharifi and Zarea (1970); Badawi (1981); Resham *et al.* (1986); Singh and Gangwar (1989). Radke and Kandalkar (1988) reported that the incubation periods varied from 3.24 days, 3.10–6.10 days, 3–7 days, 4–7 days and 5 days respectively. The differences in the incubation period was due to variations in the weather factors of different regions.

TABLE 2. Morphometric data of *Papilio demoleus* L. on Balaji Acid lime

Particulars/stage	Meaurements in "mm"				
	Minimum	Maximum	Mean	SD	SEM
Egg (Diameter)	0.91	1.06	0.99	0.061	0.719
Newly hatched caterpillar					
Length	2.04	2.60	2.38	0.237	0.075
Width	0.31	0.51	0.42	0.086	0.027
First instar larva					
Length	4.42	5.45	4.92	0.460	0.145
Width	1.52	1.75	1.64	0.096	0.030
Width of head capsule	0.50	0.80	0.69	0.119	0.038
Second instar larva					
Length	8.10	9.22	8.76	0.489	0.155
Width	2.45	2.90	2.73	0.218	0.069
Width of head capsule	0.80	1.14	1.01	0.138	0.044
Third instar larva					
Length	12.25	14.50	13.52	1.040	0.0329
Width	3.50	4.00	3.76	0.211	0.067
Width of head capsule	1.45	1.80	1.65	0.168	0.053
Fourth instar larva					
Length	22.0	44.00	41.43	2.384	0.754
Width	6.48	7.00	6.71	0.215	0.068
Width of head capsule	3.32	3.80	3.66	0.186	0.059
Fifth instar larva					
Length	38.50	44.00	41.43	2.384	0.754
Width	6.48	7.00	6.71	0.215	0.068
Width of head capsule	3.32	3.80	3.66	0.186	0.059
Pre-pupa					
Length	25.00	28.00	26.85	1.286	0.407
width	7.50	7.91	7.75	0.157	0.050
Pupa					
Length	29.00	32.00	30.61	1.207	0.382
Width	8.80	9.45	9.19	0.256	0.081
Adult moth					
Male					
Length (head to tip of abdomen)	25.50	29.00	27.43	1.249	0.395
Width	5.80	6.10	6.02	0.198	0.662
Wing expanse	87.02	91.50	89.67	1.739	0.550
Female					
Length	26.50	30.20	28.42	1.528	0.483
Width	5.90	6.40	6.26	0.239	0.076
Wing expanse	87.00	94.10	92.65	2.886	0.913

Mean of 10 samples; SD: Standard Deviation; SEM: Standard Error of Mean.

Larval development

There were only five larval instars which totally lasted for 16.90 days to 18.31 days. The average duration of first to fifth instars were 2.97, 3.27, 3.98, 3.37, and 3.99 days, respectively.

First instar larva

Newly hatched caterpillars were less spiny, cylindrical in shape, light brown to brownish black in colour with thorax thicker than rest of the body having dirty white mark on dorsal side showing resemblance to birds excreta. The newly hatched caterpillar on an average measured 2.38 and 0.42 mm length and width. The first instar larva recorded an average length and width of 4.92 and 1.64 nun respectively. The average duration of first instar larva was 2.97 days. These finding were in agreement with the reports of Atwal (1964); Resham *et al.* (1986); Ganguli and Ghosh (1967); Maheswarababu (1988).

Second instar larva

The second instar larvae were less spiny and dark brown in colour with a dirty white line present obliquely along lateral sides of the abdomen and with a break on the dorsal side. A horn—like structure was present on the dorsal side. of the last body segment. The average size of the second instar larvae (in length and width) was found to be 8.76 and 2.73 mm respectively. The average second instar larval period was found to be 3.27 days. These results were differing with the observations made by Ganguli and Ghosh (1967) who recorded 7.00 and 2.00 mm as length and width. This deviation might be due to differences in climatic factors and also due to seasonal variations from year to year.

Third instar larva

Third instar larvae were resembled the second instar larvae except in size. Third instar larvae was recorded an average length and width of 13.52 and 3.76 mm respectively. The average third instar larval period was 3.98 days with a range of 3.90 to 4.10 days. Similar observations were made by Maheswarababu (1988).

Fourth instar larva

The fourth instar larvae were black in colour with a little greenish tinge and whitish bands could be seen on meso and meta thoracic segments laterally, anterior part of abdomen and on last anal segments. Two reddish sacs or osmeteria opening in the first thoracic segment dorsally at the anterior portion. The average fourth instar larval period was found to be 41.43 mm in length and 6.71 mm in width. The average fourth instar larval period was found to be 3.37 days with a range of 3.25 to 3.46 days.

Fifth instar larva

Fifth instar larvae were yellowish green or green in colour. Brownish stripes present on eighth and ninth sternites with two semi circular yellowish bands on elevated portion of the body. Two eye-like spots were present on second thoracic segment and a horn-like structure was found on the dorsal side of the last body segment. The average length and width of the fifth instar larva was found to be 41.43 mm

and 6.71 mm respectively. The average duration of fifth instar larva was 3.99 days. Atwal (1964); Maheswarababu (1988); Asokan (1997) also recorded more or less similar observations. Fourth and fifth instar larvae had an osmeterial gland in the first thoracic segment and this organ was defensive in function. These descriptions were in agreement with those by Leslie and Berenbaum (1990); Burger *et al.* (1978) who reported that secretions produced by osmeterium contained iso-butyric acid, 2-methyl butyric acid and small quantities of methyl and ethyl esters. The average width of head capsule of *P. demoleus* during the first, second, third, fourth and fifth instars were 0.69, 1.01, 1.65, 3.66, and 3.66 mm respectively. Madansuri *et al.* (1979); Asokan (1997) observed more or less similar trends in width of head capsule.

Habits of the larva

Larval stage of the pest causes damage by feeding voraciously on tender leaves and terminal shoots. As a habit, they feed from the margin reaching the midrib. Grown up larvae even fed on mature leaves and completely defoliated the nurseries. The damage was more predominant in the nursery than in orchard trees. The habit of the larva and nature of damage was in conformity with the observations of Atwal (1964); Bhutani (1973).

Pre-pupa

Before changing to pre-pupa, the caterpillar shrunk in size and it hung from the twig with the help of a silken girdle. These were in agreement with Atwal (1964); Ayyar (1963). The pre-pupal period was observed to be 1.00 to 1.05 days with an average of 1.20 days. The average pre-pupal length and width was found to be 26.85 and 7.75 mm respectively. These observations were in conformity with Radke and Kandalkar (1988); Maheswarababu (1988); Asokan (1997).

Pupa

Pupae were naked and varied in colour from green, straw to brown, majority being green in colour with several black markings on the body. These observations were in conformity with those of Atwal (1964); Bhutani (1973); Resham *et al.* (1986). The average length and width of the pupal period was found to be 30.61 and 9.19 mm respectively. These were in conformity with the observations of Atwal (1964); Bhutani (1973); Resham *et al.* (1986). The duration of the pupal period was observed to be 8.50 days and 9.50 days with an average of 9.14 days. Ganguli and Ghosh (1967), Radke and Kandalkar (1988), Sharifi and Zarea (1970) observed similar trends in the duration of pupal period. Total life cycle from egg to adult was observed to be 29.9 and 32.61 days with an average of 31.36 days.

Adult

Adult butterflies were large and beautiful with wide wing expanse (mean of both male and female) of 9.12 cm. Head, thorax and legs were black with creamy yellow streaks

on either side and whole of abdomen. The body was covered with black and yellow hairs. Fore wings were triangular in shape while hind wings were rounded. The wings were black with yellow markings. There were two rows of parallel yellow spots along outer margins of wings and a brick red oval patch on posterior angle of the hind wing. Antennae were black and club-shaped. These descriptions were in agreement with the findings of Atwal (1964); Resham *et al.* (1986); Maheswarababu (1988). The average length, width and wing expanse of male butterfly was found to be 27.43 mm, 6.02 mm and 89.67 mm while that of female butterfly was found to be 28.42mm, 6.26 mm and 92.65 mm respectively. These were in conformity with the findings of Atwal (1964); Maheswarababu (1988); Resham *et al.* (1986). The male to female sex-ratio was found to be 1 : 2.6 on balaji acid lime.

Adult longevity

The female adults were lived longer than the male ones. The longevity of female and male was 6.75 to 6.95 and 3.75 to 4.00 days with an average of 6.86 and 3.87 days when provided with dilute honey as a food. The variation in adult longevity was in agreement with the findings of Atwal (1964). Singh and Gangwar (1989) reported the longevity female and male was 5.80 and 5.10 days.

Mating

Mating usually occurred in during the early hours of the day on tender twigs. In the act of courting, male took the initiative and searched for female. At the time of mating, the pairs touched their bodies from end to end. The average pre-mating and mating period were found to be 12.10 hours and 1.80 hours. Atwal (1964), Radke and Kandalkar (1988) also reported similar observations.

Pre-oviposition and oviposition period

The females were found to lay eggs within one to two days after mating and continued for one to five days. Pre oviposition period ranged from 1.45 to 1.65 days with an average of 1.56 days. The oviposition usually took place during the night. The average number of eggs laid by a gravid female moth was 119 eggs. The average oviposition period lasted for 4.32 days with a range of 4.10 to 4.45 days. Maheswarababu (1988); Atwal (1964) reported similar observations. The post oviposition was found to be 4.00 to 4.75 hours with an average of 4.09 hours. This was in agreement with the observations of Maheswarababu (1988), Radke and Kandalkar (1988).

Based on the observations made in this study it is concluded that the average incubation period lasted for 3.65 days with a range of 3.50 to 3.75 days. The average first, second, third, fourth and fifth instar larvae lasted for 2.97, 3.27, 3.98, 3.37 and 3.99 days respectively. The pre-pupal period lasted for 1.00 to 1.05 days while the pupal period lasted for 8.50 to 9.50 days. The average total life cycle of citrus butterfly lasted 31.3 days with a range of 29.90 to 32.61 days.

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New species of Bumblebee (Hymenoptera: Apidae: Bombinii) recorded from North–West Himalaya

Avdhesh Kumar*,† and Alexander Lall

School of Entomology, St. John's College, Agra 205002, India

ABSTRACT: The new species *Bombus (Bombus) manaliensis* sp. nov. of tribe *Bombinii* from North–West Himalaya is described based on male genitalia. The female is described based on sting sheath and pubescence of body.

KEYWORDS: N. W. Himalaya, *Bombus*, new species, *manaliensis*

INTRODUCTION

Bingham (1897) treated the members of Bombidae under the Heterogenous assemblage of family Apidae. He treated 24 species of Bumblebees under a single genus *Bombus* Latreille. Our knowledge of Bumblebee (Bombidae) fauna of Europe and Asia is derived from the efforts of Richards (1927, 1929, 1968), who studied very extensively the bumblebees of British Islands. In 1927 while studying the British bumblebees who described in considerable detail morphological characters of taxonomic importance in Bombidae. Prior to this publication in majority of cases main emphasis for the identification of different species was laid on the colour patterns of the pubescence covering different parts of the body. Richards (1927) laid greater emphasis on the structure of the sting sheath and the wide range of variations in the male genitalia, which help in authentic placement of different species. Vogt (1911) was perhaps the first person, who divided genus *Bombus* into subgenera on the basis of the characters of male genitalia. His work was further amplified by Kruger (1920) who divided the genus *Bombus* into primary divisions (1) *Odontobombus* (2) *Anodontobombus*. Following their examples Richards (1927) was able to describe a large number of subgenera like, *Odontobombus* Kruger, *Hotobombus* Vogt, *Subterraneobombus* Vogt, *Lapidariobombus* Vogt, *Cullumanobombus* Vogt, *Pratobombus* Vogt, while studying British bumblebees.

*Corresponding author
†Present address: Department of Entomology, College of Agriculture, GBPU&T, Pantnagar, India.

Frison (1926, 1927, 1933, 1935) paid special attention to the study of North American bumblebee fauna. Milliron (1961, 1971, 1973a,b) produced a monumental work on the bumblebee fauna of Western hemisphere in the form of a monograph. Milliron (1961) system of classifying the bumblebees into three genera has not been widely accepted and most of those working with the group appear to be reasonably satisfied with the subgeneric classification of the single bumblebee genus *Bombus* Latreille, 1802 as summarized by Richards (1968), which is based on similarity in the characteristics of the male genitalia. Richards (1928) described some species of *Bombus* from Ladakh and other parts of Himalaya. Gupta (1960) studied some Hymenopterans collected by Prof. Mani's Third Entomological Expedition to North-west Himalaya. He recorded two species of *Bombus* for the first time from higher reaches of Kullu and Lahaul Spiti valleys. Williams (1991, 1998) did extensive work on the Indian bumblebees, he reported several species from Northern Himalaya. We have followed the latest classification of Bees by Michener (2000) who treated family Bombidae a tribe (Bombinii) under Apidae sub family of Apidae.

MATERIALS AND METHODS

Specimens were collected by the first author with the help of sweeping net from different parts of North-west Himalaya during 1992–1998. Specimens were pinned and stored in wooden boxes with Para dichlorobenzene. The mouth parts, antenna, legs, male genitalia, 7th, 8th gastral (metasomal) sternites and female sting sheath were treated with 10% KOH for about 5–7 days at 35 °C room temperature and after dehydration mounted in DPX.

RESULTS AND DISCUSSION

Bombus (Bombus) manaliensis sp. nov. male (Plate I, II and III)

Head

Eyes not swollen. Ocelli separated by nearly three diameters from eyes, located on postocular line. Frons densely covered with stiff black setae. Antennal segments 3 : 4 : 5 : 8 = 6 : 4 : 5 : 5, third antennal segment almost as long as the fifth. Outer margin of mandibles covered with dense, long beard; setae a long the base directed towards the teeth almost over the distal setae; ventral tooth large, broad; dorsal tooth much smaller; a prominence in the middle separating the proximal malar part from the distal. Malar space transverse, longer than antennal segment three, malar space 0.56 mm long and 0.72 mm wide, first labial palpus 2.56 mm long. Glossa 4.88 mm long.

Thorax

Ventral surface covered with long, dense whitish pubescence. Mid-basitarsus relatively broad, parallel sided, fringes short. Hind tibia slightly convex, somewhat hollowed out towards dorsal margin; proximal quarter outer margins bare, covered with blackish

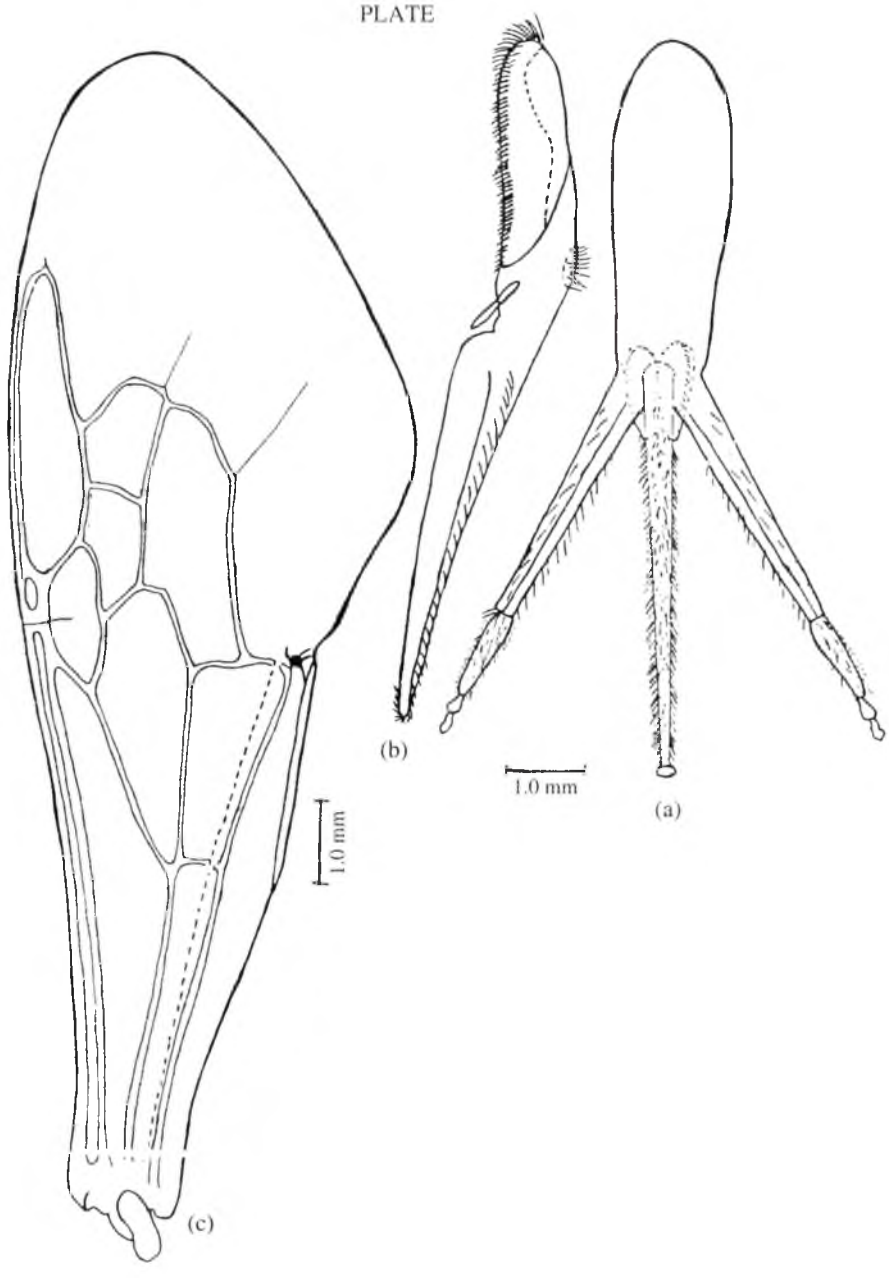

PLATE I. Fig. (a): Proboscis of male with labrum, (b). Labium, (c). Fore wing.

PLATE

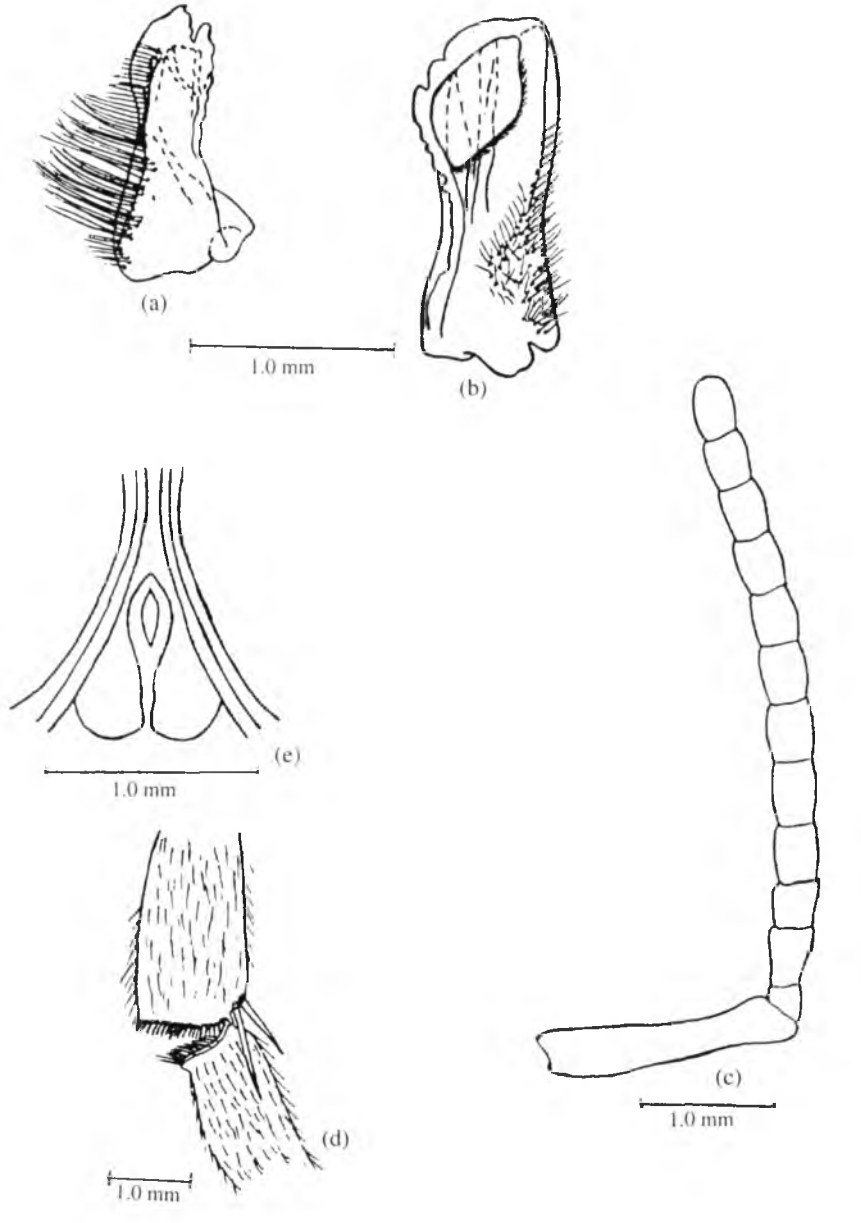

PLATE II. Fig. (a) Manble of male, (b) Mandible of female, (c) Antenna of male, (d) Pollen basket of female, (e) Sting sheath of female.

PLATE

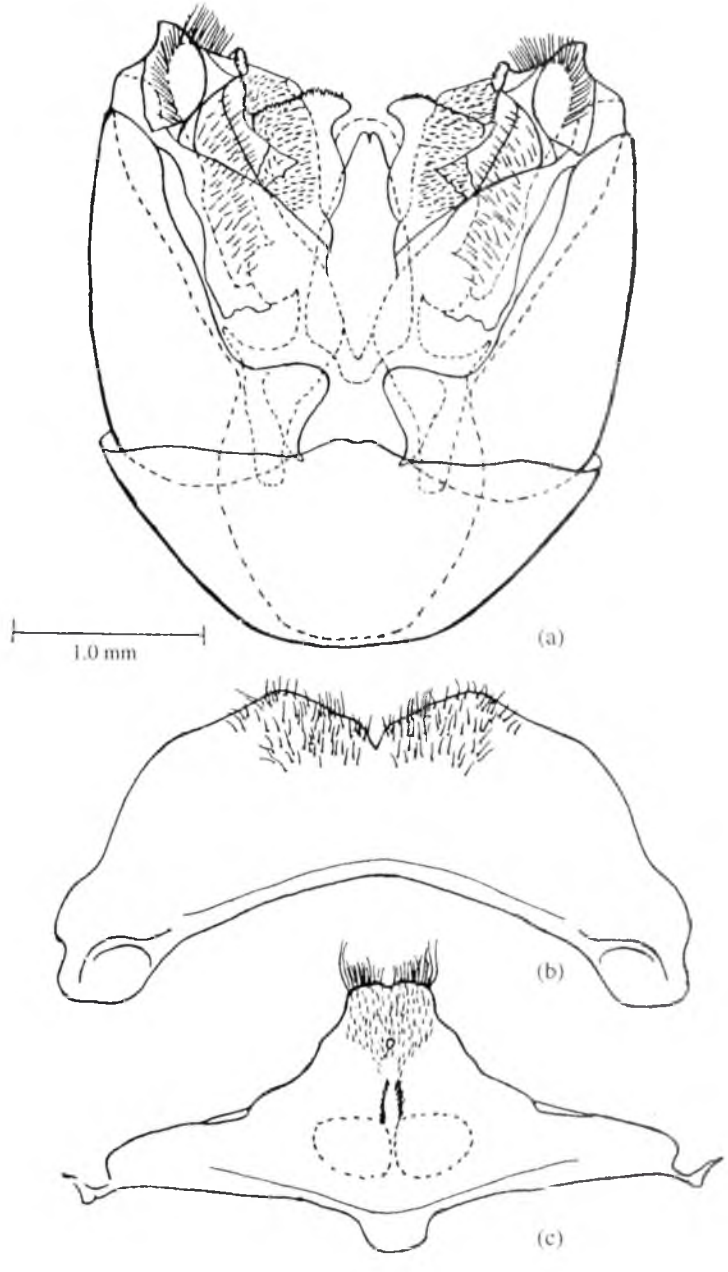

PLATE III. Fig. (a) Male genitalia, (b)–(c), 7th and 8th gastral sternites of male.

setae, fringes especially the dorsal long. Hind- basitarsus with distal angle just acute, fringes short; marginal cell of wing 3.76 mm long.

Abdomen

First six gastral (metasomal) sternites black with their posterior margins pale, sparsely covered with long delicate pale setae; fourth and fifth sternites bare medially. Gastral sternite 6th with margin considerably recurved, thickened with a central post apical fringe. Gastral sternite 7th (Plate III, B) transverse, posterior margin rounded with central emargination, long bristles on either side, no fenestrae. Gastral sternite 8th (Plate III, C) broad, trapiiziform, sides strongly converging, apex weakly emarginated with corners angled, tufts of long bristles arising near these angles, no fenestrae.

Male genitalia (Plate III, A)

Cardo broad, oval, compressed at base. Stipes broad at base, outer margin somewhat straight, inner margin slightly impressed medially, setae absent. Squama quadrate, transverse with anterior sub-membranous inner projections separated from main lobe by a deep impression, main lobe expanded inwards in more or less trumpet shaped projection, outer margin concave medially, inner margin convex, apex with pointed setae medially. Lacinia projecting little beyond squama, apex transverse strongly produced inwards into a process ending in a slight upturned hook, hind margin rough with dense long setae apically. Sagitta narrow in dorsal view, undulating and widely flaring at apex, in side view very wide, deeply emarginated below just above base, apex wide, curved outwards, setae dense, short apicomedially. Spatha widened basally, narrow apically, apex truncate, notched, obtuse at base, anterior to the articulation with sagitta. Volsellar region with short, sparse setae.

Female (Plate II, B, E, and D)

Head

Ocelli separated by nearly three diameters from eyes, located just in front of postocular line, all ocelli almost in a straight line with the middle one inconspicuously towards the frons. Frons shiny black with numerous punctures and well-defined unpunctured area, a band of dense fine punctures along inner margin of eyes. Antennal segments 3 : 4 : 5 := 6 : 4 : 5, segment three twice as long as wide. Clypeus short, convex, densely punctured, epecially apical impression. Malar space transverse, 0.60 mm long and 0.60mm wide, longer than antennal segment three, shining with minute irregular punctation. Mandibles sharply curved, spatulated with a deep incision and distinct sulcus obliquus. Maxilla elongated, outer margin fringed with distinct long setae proximally, minute setae distally; maxillary palpi two segmented, second segment nearly twice as long as stipes, produced apically into an acute point, not curved inwardly. Labium with prementum cylindrical elongated, slightly narrow posteriorly bearing four segmented labial palpi, first segment of labial palpi 3.04 mm long, nearly three times as long as second segment, third and fourth labial segments minute,

combined length less than one third the length of second segment. Glossa elongated, 6.88 mm long, extending beyond the labial palpi, one and a half times as long as the first segment of labial palpi.

Thorax

Densely pubescent, posterior plates of the prosternum touch each other medially, propleuron with distinct shallow punctations, minute setae arising from the base of the pits. Wings fused, dense infuscate, darker along costal margin, pterostigma dark brown, submarginal cell divided into two parts by transector, posterior margins of first sub-marginal distinctly curved anteriorly, marginal cell 3.40 mm long, elongated gradually narrowing apically. Mid-basitarsus with posterior apical angle obtuse. Hind tibia with corbicular surface bare, weakly reticulated, dorsal inner apical angle distinctly produced, volume of pollen basket 1.38 cubicmm. Hind basitarsus with dorsal angle acute, surface densely pubescent, bristles short.

Abdomen

Gastral sternite 6th feebly sculptured, shining. Sting sheath with outer thickenings narrow but broadened dorsally, inner thickenings wide and twice emarginated. Gastral sternites distinctly punctated, posterior margin of sternites with fringe of blackish setae.

Colouration

Head black with dense black and long setae in male, head black with black short setae in female. Anterior part of thorax with silvery white wide band of pubescence in female, relatively dirty white in male; between the bases of wings a band of black pubescence followed by obtusely triangular patch of white pubescence on the metathorax. First abdominal segment in both sexes covered with white backward directed pubescence, second abdominal segment covered with orange yellowish pubescence followed by a black band of pubescence on the third tergite; fourth, fifth and sixth tergites with dark orange red pubescence in female, relatively lighter in male, under surface of thorax in female with black pubescence and pale white pubescence in male.

Size

Male body length 14–17 mm (64). Female body length 16–20 mm (20). Workers body length 12–14 mm (4).

Type

Holotype

Male, Manali (Distt. Kullu, H.P.) Coll. Avdhesh Kumar 26.X.1992. (mouth parts, wings, legs, 7th and 8th gastral sternites, male genitalia on slides)

Paratype

7 males, 4 females, Bharmour (Distt. Chamba, H.P.) Coll. Avdhesh Kumar 23.X.1992; 38 males, Manali, 22 females, Solang, 4 males, Gulaba (Distt. Kullu, H.P.) Coll. Avdhesh Kumar 14.X.1994; 8 females, Manali, 6 females, Solang, 10 females, Manali, (Distt. Kullu, H.P.) Coll. Avdhesh Kumar 20.X.1996.

Affinities

Bombus (Bombus) manaliensis sp. nov. superficially comes close to *Bombus (Alpigenobombus) tunicatus* (Smith) in the over all colour patterns of body except that the colour of pubescence on second abdominal segment is dark rusty in the new species and dirty whitish in *tunicatus*. Besides the former can readily be differentiated from *tunicatus* by the differences in 7th and 8th gastral (metasomal) sternites of male. In tunicatus the 7th gastral sternite is broadly rounded but it is deeply notched in *manaliensis*; the distribution of setae in the *tunicatus* is restricted only to two patches on the posteriolateral margins as compared to almost continuous single patch on the either side of the notch in *manaliensis* and 8th gastral sternite in *tunicatus* also exhibit marked differences. Lacinia in both species is entirely different, the distribution of setae on lacinia are restricted to only inner margins in *tunicatus* whereas in *manaliensis* it is almost uniformly distributed all along the margin.

Etymology

The species is named after the locality from which it was collected.

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A survey of spiders (Araneae: Araneidae) of Jaldapara Wildlife Sanctuary, West Bengal, with description of a new *Zilla* species

S. Saha and D. Raychaudhuri*

Entomology Laboratory, Department of Zoology, University of Calcutta, 35, Ballygunge Circular Road, Kolkata 700019, India
Email: dinendrarc@rediffmail.com

ABSTRACT: The present paper reports 22 orb-weaving spiders (Araneidae) from Jaldapara Wildlife Sanctuary. Of these one species, *Zilla globosa* is described as new to science, while two species, *Araneus nympha* Simon and Poltys bhabanii (Tikader) are reported as new records from West Bengal and two species, *Leucauge bengalensis* Gravely and *Neoscona rumpfi* (Thorell) as new from the district Jalpaiguri.

KEYWORDS: Araneid spiders, new species, new records, Jaldapara Wildlife Sanctuary

INTRODUCTION

A close scrutiny on the study of Biswas and Biswas (1992) on the spider fauna of West Bengal (Araneae) reveals that sustained survey is wanting for Jaldapara Wildlife Sanctuary, Jalpaiguri, West Bengal. In a study on the spiders of the sanctuary from September 2001 to July 2004 we could record 22 species of true orb-weaving spiders (Araneidae) distributed over 11 genera. We could not determine the species status of *Nephila* Leach because of its immaturity. Among the other 21 species, *Zilla globosa* is described as new to science while *Araneus nympha* Simon and *Poltys bhabanii* (Tikader) are reported as new records from West Bengal and *Leucauge bengalensis* Gravely and *Neoscona rumpfi* (Thorell) as new reports from the district Jalpaiguri.

Recorded spider samples are in the collection of Entomology Laboratory, Department of Zoology, University of Calcutta.

*Corresponding author

MATERIAL AND METHODS

Study area

Jaldapara Wildlife Sanctuary, Jalpaiguri, West Bengal, north-east India, is situated between latitudes 25°58′ and 27°45′ north and longitudes 89°08′ and 89°55′ east. The total area of the sanctuary is 216.51 km^2, comprising mainly of humid tropical rain forests.

Collection and preservation of the spider samples were done following Tikader (1987). These were recorded during the period October 2001–November 2002. The materials were studied using a stereozoom binocular microscope, model Zeiss SV11. All the measurements are in millimeters, made with an eyepiece graticule.

RESULTS

Details of the araneid spiders recorded in this study (except the new species) are given in Table 1.

Description of the new species

Genus: *Zilla* stat. C. L. Koch, 1834
Zilla C. L. Koch, 1834, Arachn. Inaect. : 124

Generic diagnosis

Cephalothorax moderately convex, slightly pubescent. Eyes small; anteromedians closer to anterolaterals; distance between anteromedians and posterolaterals equal to distance between posteromedians and posterolaterals; ocular quad rectangular, longer than wide; anterior row recurved; posterior row straight or slightly recurved. Maxillae long, basally narrow, distally broad and scopulate. Labium wider than long. Legs long, strong, clothed with hairs, spines and bristles. Male palp long, with a large erect spine. Abdomen short, oval, subelliptical, depressed, pubescent, with a silvery lusture. Epigynum with or without scape.

Distribution

America, Asia, Europe.

Type species

Aranea diodia stat. Walckenaer, 180.
Zilla globosa sp. nov.

Species diagnosis

General colour

Cephalothorax and legs yellowish, abdomen greyish with chalk white reticulation.

TABLE 1. Details of the araneid spiders recorded from Jaldapara Wildlife Sanctuary, West Bengal

Species	Specimens examined	Locality	Distribution recorded earlier (see below for explanation of abbreviations and references)
***Araneus nympha* Simon, 1889	2♀	Bengdaki	WB, HIM CHN, PAK
Argiope minuta Karsch, 1879	1♀	Jaldapara	ML, WB, BGD; CHN, JPN, KOR, SIN, THA
Argiope pulchella Thorell, 1881	1♀	Jaldapara	AI, AS, GJ, MP, SK, WB BGD, CHN, IDN, MAL, MYN, PAK, SIN
	2♀	Sissamara	
	5♀	Dhoidhoighat	
	1♀	TEC	
	1♀	Bania	
	1♂	Chilapata	
	1♀	CCLine	
	1♀	Hasimara	
Cyclosa bifida (Doleschall, 1859)	1♀	Jaldapara	ML, SK, WB, MAL, NGA, PHL, SLK
Cyclosa quinqueguttata (Thorell, 1881)	1♀	Malangi	SK, WB, BHU, CHN, MYN, TAN
Cyclosa simoni (Tikader, 1982)	1♀	Sissamara	SK, WB
Cyclosa spirifera Simon,1889	1♀	Bengdaki	WB
	1♀	Malangi	
	2♀	Dhoidhoighat	
Cyrtophora moluccensis (Doleschall, 1857)	4♀	Dhoidhoighat	AI, NI, KA, KR, MP, WB, AML, CHN, JPN, MYN, NPL, SLK
Gasteracantha diadesmia Thorell, 1887	1♀	Bania	AI, NI, ML, SK, WB, BGD, CHN, JPN, MYN, THA, PHL
	1♀	Mendabari	
	2♀	CCLine	

TABLE 1. Contd...

Species	Specimens examined	Locality	Distribution recorded earlier (see below for explanation of abbreviations) and references
Gasteracantha kuhlii C.L.Koch, 1838	1♀	TEC	AI, NI, AS, BI, ML, SK, WB, BHU, CHN, IML, JPN, MYN, PHL
	1♀	Sissamara	
	1♀	Kunjanagar	
	1♀	NWC	
	1♀	CCLine	
Gea subarmata Thorell, 1890	1♀	Jaldapara	WB, BGD, JPN, IDN, MYN, NGA, PHL
Leucauge celebesiana (Walckenaer, 1841)	2♀	Dhoidhoighat	AS, MH, ML, SK, WB, CHN, MYN, SLK
Leucauge decorata (Blackwall, 1864)	2♀	Sissamara	AS, BI, GJ, KA, KR, ML, TN, UP, WB, AUS, BGD, CHN, HKG, JPN, MYN, NGA, SOL, SLK, TAN, THA, UGD
	1♂2♀ (Immature)	TEC	
	1♀	Hollong	
	4♀	Siltorsa	
	6♀	Bania	
	1♀	Chilapata	
	3♀	CCLine	
Leucauge fastigata (Simon,1877)	1♀	Jaldapara	KR, OR, UP, WB, MYN, SLK
	3♀	CCLine	
	1♀	Mendabari	
**Leucauge bengalensis* Gravely, 1921	5♀	Jaldapara	WB
Neoscona mukerjei Tikader, 1980	3♀	Malangi	MH, ML, WB, BGD
	1♂ (immature)		
	1♀	TEC	
	1♂	Bania	
	1♂	Mendabari	

TABLE 1. Contd...

Species	Specimens examined	Locality	Distribution recorded earlier (see below for explanation of abbreviations) and references
Neoscona nautica (C.L.Koch, 1875)	1♀	Moiradanga	GJ, MH, ML, WB, AMR, BGD, CHN, JPN, MYN, PAK
	2♀	Malangi	
	1♀	Sissamara	
	1♀	Dhoidhoighat	
Neoscona rumpfi (Thorell, 1887)	1♀	TEC	AP, MH, OR, TN, WB, AML, IML, MYN, PAK, SLK
Parawixia dehaanii (Doleschall, 1859)	3♀	Jaldapara	KA, SK, WB, AML, IML, JPN, IDN, MYN, NGA, PHL, POL
	1♀	TEC	
	1♀	Kunjanagar	
	1♀	NWC	
	1♀	CCLine	
****Poltys bhabanii*** (Tikader, 1970)	1♀	TEC	SK, WB

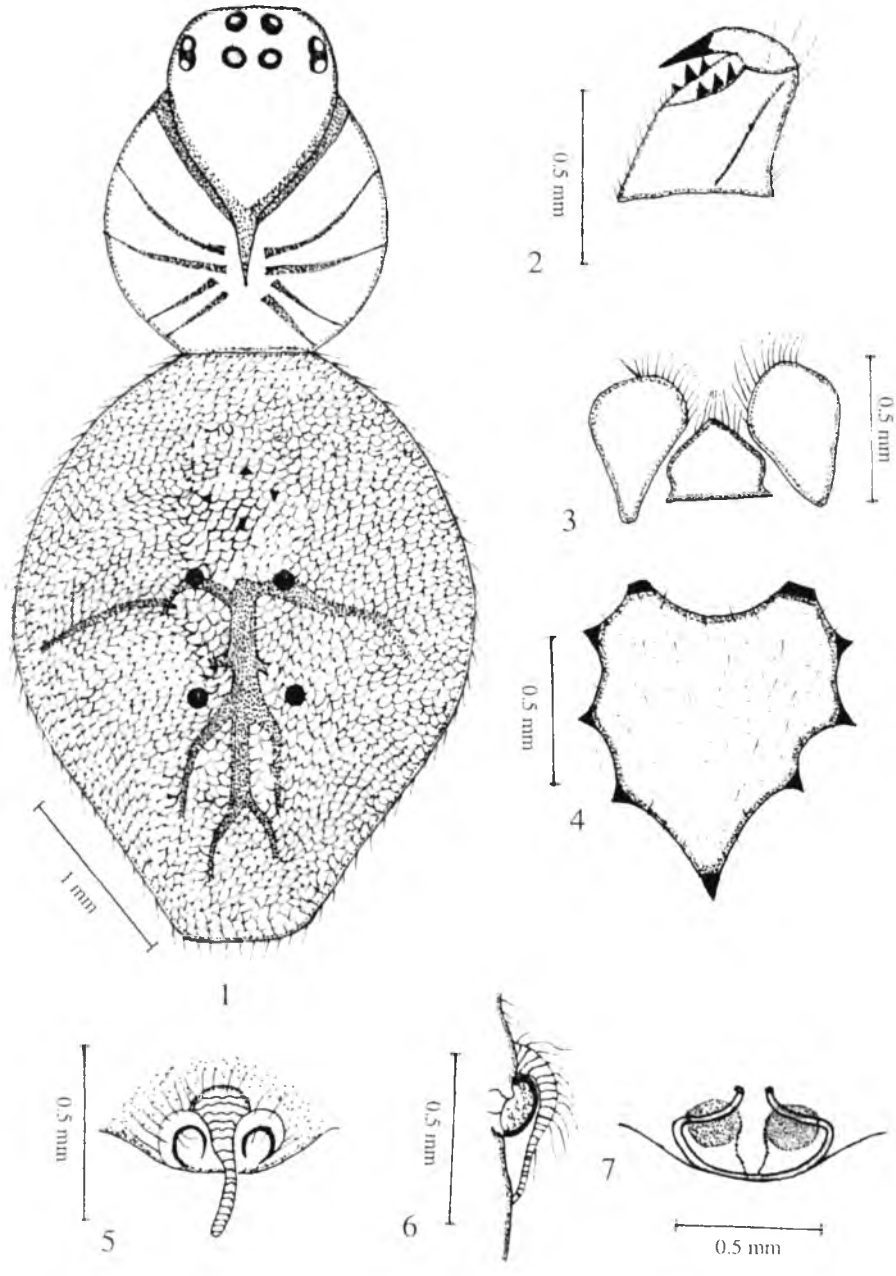
*New record from the distinct Jalpaiguri, **New record from West Bengal AI, Andaman Islands; AP, Andhra Pradesh; AS, Assam; BI, Bihar; GJ, Gujarat; KA, Karnataka; KR, Kerala; MH, Maharashtra; ML, Meghalaya; MP, Madhya Pradesh; NI, Nicobar Islands; OR, Orissa; SK, Sikkim; TN, Tamil Nadu; UP, Uttar Pradesh; WB, West Bengal; AML, Auvtro-Malayasia; AMR, America; AUS, Australia; BGD, Bangladesh; BHU, Bhutan; CHN, China; HIM, Himalaya; HKG, Hong Kong; IDN, Indonesia; IML, Indo-Malayasia; JPN, Japan; KOR, Korea; MAL, Malayasia; MYN, Myanmar; NGA, New Guinea; NPL, Nepal; PAK, Pakistan; PHL, Philippines; POL, Polynesia; SIN, Singapore; SLK, Sri Lanka; SOL, Solomons; TAN, Taiwan; THA, Thailand; UGD, Uganda

Female (holotype)

Total length 5.20; carapace length 1.93, carapace width 1.63; abdomen length 3.30, abdomen width 2.60; legs as in Table 2.

Cephalothorax (Fig. 1) globose, anteriorly narrow, posteriorly rounded, slightly longer than wide, cephalic region slightly raised with deeply distinct cervical furrows; thoracic region with indistinct longitudinal groove and distinct radii; eyes pearly white, basally ringed with black bands, laterals subequal, contiguous, anteromedians smaller than posteromedians, ocular quad longer than wide, rectangular; anterior row strongly recurved, posterior row nearly straight; chelicerae (Fig. 1) yellow, long, each margin with 3 teeth, fangs reddish, strongly curved; both maxillae and labium (Fig. 3) pale, anteriorly strongly scopulate; maxillae long, basally narrow, distally truncate; labium

Zilla globosa **sp. nov.**



FIGURES 1. –7 *Zilla globosa* sp. nov., female holotype.
Figs. 1–6: *Zilla globosa* sp. nov., female hologype; 1: Whole body; 2: Chelicerae; 3: Maxilla and labium; 4: Sternum; 5&6: Epigynum; 7: Internal genitalia.

TABLE 2. Length of legs of ♀ holotype of *Zilla globosa sp. nov.* (in mm.)

Leg	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	2.55/2.55	0.5/0.5	2.9/2.9	1.4/1.4	2.0/2.0	9.35/9.35
II	2.0/2.0	0.5/0.5	2.5/2.5	1.65/1.65	1.35/1.35	8.00/8.00
III	1.65/1.65	0.6/0.6	1.25/1.25	1.5/1.5	0.45/0.45	5.45/5.45
IV	2.45/2.45	1.2/1.2	2.25/2.25	2.65/2.65	0.95/0.95	9.50/9.50

wider than long, basally with a lateral notch, distally convex and produced; sternum (Fig. 4) pale, heart shaped, with anterior margin concave, posteriorly narrowed and produced, clothed with hairs and black spines; legs long, strong, clothed with bristle like black hairs and few spines; leg formula 4123.

Abdomen (Fig. 1) elongate, oval, slightly overlapping cephalothorax, clothed with fine hairs and brown spines; dorsum medially with greyish lines and two pairs of sigillae; venter greyish with scattered white reticulation; epigynum and internal genitalia as in Figs. 5, 6 and 7.

Material examined

Holotype, ♀, 29.4.2002, Hollong, Jaldapara Wildlife Sanctuary, Jalpaiguri, West Bengal, India; Coll. S. Bhattacharjee (Regn. No. EZC 0001-03).

Distribution

India: West Bengal (known only from the type locality).

Remarks

Presence of epigynal scape brings the present species close to *Zilla diodia* (Walckenaer) known from England, *Z. astridae* (Strand) and *Z. sachalinensis* (Saita) from China. However, the epigynum together with its scape in the present species appear much different. Therefore, the species, as such is described as new to science.

Etymology

The species name refers to the globose nature of cephalothorax.

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A new species of *Listrognathus (Listrognathus)* Tschek (Hymenoptera: Ichneumonidae) from Kerala, India

K. Sudheer* and T. C. Narendran

Systematic Entomology Laboratory, Department of Zoology, University of Calicut, Kerala 673635, India

ABSTRACT: A new species of *Listrognathus*, *viz.*, *Listrognathus* (*Listrognathus*) *keralensis* is described from Kerala, India. A key to the Indo-Australian species is also provided.

KEYWORDS: Ichneumonidae, *Listrognathus (Listrognathus) keralensis*, new species, India

INTRODUCTION

The genus *Listrognathus* Tschek belongs to the tribe Goryphiini of the subfamily Mesosteninae. The genus *Listrognathus* (*Listrognathus*) Tschek is represented by eighteen species in the Indo-Australian region (Gupta, 1987). Gupta and Kamath (1967) studied the Indian species and Kamath (1967) studied the species from Burma, China and Philippines. In the present paper a new species, *Listrognathus* (*Listrognathus*) *keralensis* sp. nov. is described and a key to the Indo-Australian species is provided. The type of the new species is deposited in the Department of Zoology, University of Calicut for the time being but eventually will be transferred to the Zoological Survey of India, Kozhikode, Kerala.

MATERIALS AND METHODS

The collections were made using Sweep net, as described by Narendran (2001). The dried specimen was mounted on a rectangular card. The mounted specimen was held on No. 3 Asta insect pins of size 38 mm × 0.55 mm. The specimen was observed and studied under Olympus (Japan) microscope and the figures were drawn using M3Z WILD stereozoom (Switzerland made) microscope.

*Corresponding author

***Listrognathus* (*Listrognathus*) *keralensis* sp. nov. (Figs 1–5)**

Female

Total length (including ovipositor): 17.06 mm.

Pubescence

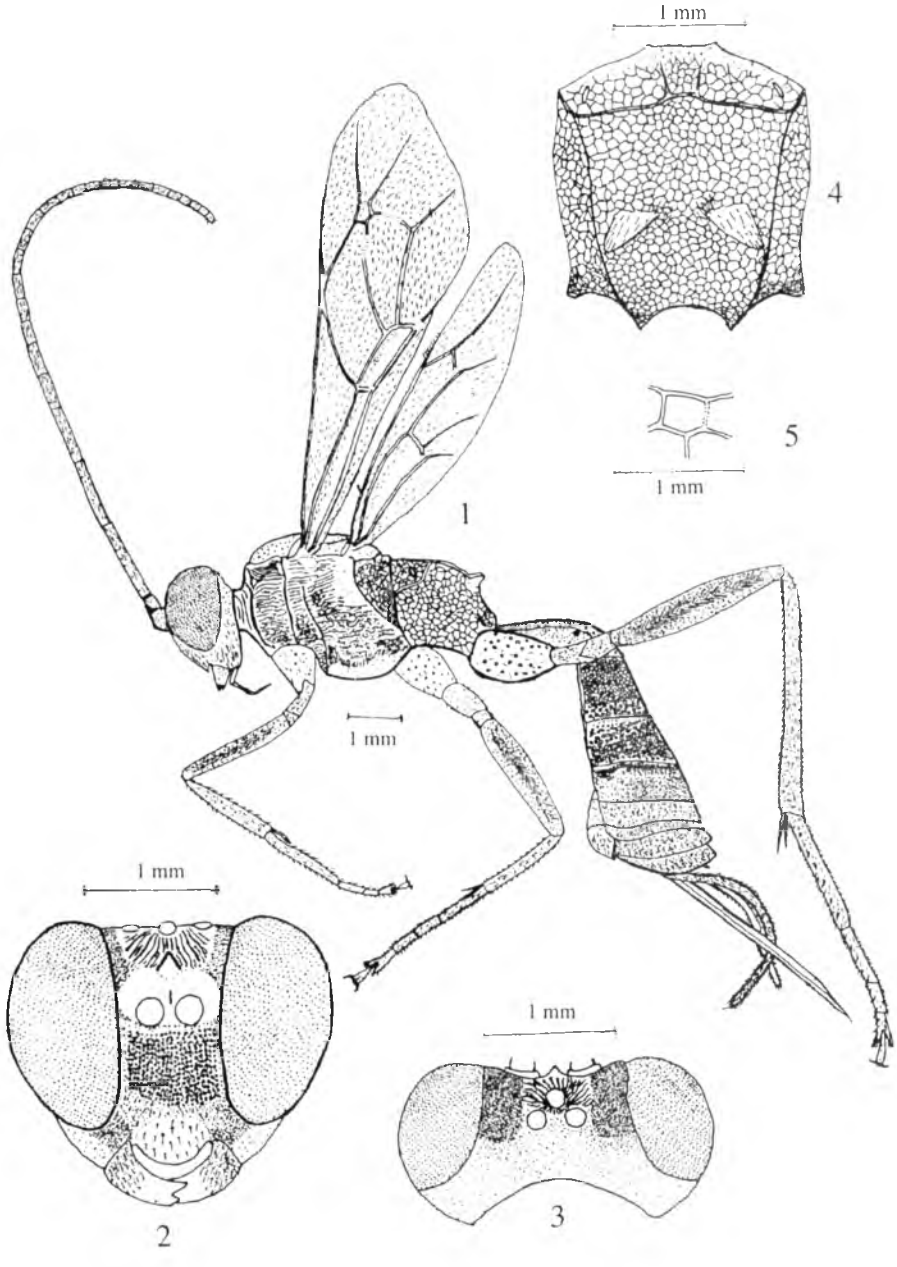
Body covered with closely arranged fine white hairs; hairs longer on propodeum.

Head

In front view HL = 1.82 mm and HW = 2.44 mm (Fig. 2); in dorsal view HL = 0.89 mm and HW = 2.41 mm (Fig. 3); face closely and distinctly punctate, punctures running into striations; clypeus sparsely and shallowly punctate, evenly convex, apical margin impressed; mandibles with close, shallow punctures, apex smooth; mandibular teeth unequal in shape; malar space granulose, 0.75*x* basal width of mandible (Fig. 1); frons (Fig. 2) below ocellar triangle with oblique and irregular striations, side of frons closely punctured; frons with a median horn, 0.3*x* as long as scape of antenna; vertex with distinct fine punctures; interocellar area with striations; interocellar distance 0.4*x* ocellocular distance, 2.0*x* distance between median and lateral ocelli; antenna with 27 segments; length of scape equal to its width; first flagellar segment 8x as long as its width at apex and 1.14*x* as long as second; temple with fine distinct punctures; occipital carina complete, joining well raised hypostomal carina at an acute angle.

Thorax

2.19*x* as long as width between tegulae; pronotum (Fig. 1) longitudinally striate; pronotal shoulder acute, collar evenly curved, at its lower margin without any tooth-like projection; epomia strong, not reaching upper margin of pronotum; mesoscutum with fine, evenly distributed punctures; notaulus distinct and sharp, extending beyond middle of mesoscutum; scutellum with distinct scattered punctures, interstices between punctures smooth and shiny; lateral carina of scutellum confined to base; metascutellum smooth and shiny; propodeum (Fig. 4) strongly reticulate, basolateral area finely punctate, with striations, basal carina complete, apical carina represented by propodeal apophyses; spiracle oval; mesopleuron rugoso-punctate, speculum smooth with sparse, scattered punctures, sternaulus distinct, extending to base of mid coxa, area beneath sternaulus punctate with interstices smooth; prepectal carina extending 0.75*x* height of mesopleuron; metapleuron strongly reticulate, juxtacoxal carina absent; wings clear hyaline; FWL = 9.09 mm; FWW = 2.73 mm; HWL = 6.36 mm; HWW = 2.09 mm; areolet (Fig. 5) pentagonal, 7*x* as high as width of bordering veins, intercubitii parallel, second intercubitus faint; nervulus slightly basad of basal vein; nervellus intercepted below its middle; hind wing with one basal and eight apical hamuli; legs with fine striations except hind coxa deeply punctate.



FIGURES 1–5:. *Listrognathus keralensis* sp. nov.: 1. Body profile; 2. Head – Front view; 3. Head–Dorsal view; 4. Propodeum; 5. Areolet.

Abdomen

Length of first tergite 1.3*x* length of second tergite and 2.19*x* its width at apex; first tergite shiny with closely arranged fine punctures, median dorsal carinae present; second and third tergites coarsely punctate; fourth and following tergites with fine, shallow punctures; length of ovipositor beyond apical depth of abdomen = 4.36 mm; ovipositor subcylindrical, its dorsal valve depressed between nodus and apex, without a median carina, its lower valve with teeth.

Colour

Black. Outer orbits of face, clypeus except extreme apex, orbital stripes on frons, base of mandibles, pronotal collar, tegula, scutellum, metascutellum, subtegular ridge, base of hind wing, hind margin of metanotum, propodeal apophyses, broad band on apices of first, third and seventh tergites and an interrupted band on apex of second tergite with yellow markings; fourth to eleventh flagellar segments with white dorsal stripe; coxa, trochanters, and femora of all legs orangish yellow; tibia and tarsal segments brownish black.

Male

Unknown.

Host

Unknown.

Distribution

India (Kerala).

Materials examined

Holotype: 1F, India: Kerala, Calicut University Campus, K. Sudheer, 9.x.2002.

Discussion

This new species is similar to *Listrognathus* (*Listrognathus*) *confracta* Gupta and Kamath in (1) the absence of a tooth-like projection on the lower margin of pronotum, (2) strongly striated frons and (3) size of frontal horn. The species differs from *L. (L.) confracta* in the following characters: (1) Mandibular teeth unequal (In *L. (L.) confracta* teeth equal in size); (2) Lower end of occipital carina joining well raised hypostomal carina at an acute angle (In *L. (L.) confracta* lower end of occipital carina joining well raised hypostomal carina at more than a right angle) and (3) Pronotal shoulders acute (Pronotal shoulders obtuse in *L. L. confracta*).

This new species is also similar to *L. (L.) nigriabdominalis* Gupta and Kamath in having (1) unequal mandibular teeth, (2) lower end of occipital carina joining

hypostomal carina at an acute angle, (3) occipital carina complete above and (4) apical carina of propodeum incomplete. *L. (L.) keralensis* sp. nov. differs from *L. (L.) nigriabdominalis* in the following characters: (1) Lower margin of pronotum without a tooth-like projection (Tooth-like projection present in *L. (L.) nigriabdominalis*); (2) Dorsal valve of ovipositor without a median carina (Dorsal valve of ovipositor with a median carina in *L. (L.) nigriabdominalis*); (3) Metascutellum yellow; first, third and seventh abdominal segments with apical yellow bands (Metascutellum black and abdominal segments wholly black in *L. (L.) nigriabdominalis*) and (4) Vertex with distinct, fine, evenly distributed punctures (Vertex coarsely punctate, medially obliquely striated behind in *L. (L.) nigriabdominalis*).

Key to the Oriental species of *Listrognathus* (*Listrognathus*) Tschek

1. Frons smooth and polished 2

   Frons strongly striated 8

2. Oculo-ocellar area with fine punctures and irregularly longitudinally rugose; base of scutellum yellow, apex largely black ..... *L. (L.) laevifrons* (Cameron)

   Oculo-ocellar area mat to smooth, sometimes with scattered punctures; scutellum largely yellow 3

3. Pronotal collar without a tooth-like prominence on its lower margin 4

   Pronotal collar with a distinct tooth-like prominence on the lower margin 5

4. Frontal horn large sized, provided with a small accessory horn at base behind; apical carina of propodeum in the form of a strong ridge between apophyses ..... *L. (L.) flavicornis* Kamath

   Frontal horn large but not provided with an accessory horn at base behind; apical carina of propodeum broadly interrupted in middle ..... *L. (L.) heinrichi* Kamath

5. Notauli distinctly impressed, extending to middle of mesoscutum; lateral carina of scutellum with yellow markings 6

   Notauli faintly indicated on mesoscutum; lateral carina of scutellum without yellow markings 7

6. Frontal horn moderately large, bifurcate or cleft above, never with an accessory horn; mesoscutum coarsely punctate; occipital carina interrupted in middle ..... *L. (L.) bifida* Kamath

   Frontal horn small to medium sized, sometimes with a dorsal line or an accessory horn, not bifurcate; mesoscutum rugoso-punctate; occipital carina complete ..... *L. (L.) spinifrons* (Cameron)

7. Fore femur and tibia yellow but with dark brown lines (sometimes tibia fuscous); hind tibia yellow with a basal and an apical black band; base of fifth tarsi of hind leg black ..... *L. (L.) mobilis* (Tosquinet)

Fore femur and tibia brownish yellow but femur lined with brown above; hind tibia yellow with only apical black band; base of fifth tarsi of hind leg yellow... ..... *L. (L.) pallidinerva* (Cameron)

8. Notauli weakly impressed on mesoscutum; face finely punctate; propodeum with apical transverse carina complete; first three abdominal segments basally brown; hind tibia largely black ..... *L. (L.) sauteri* Uchida

Notauli sharply impressed on mesoscutum; face strongly punctate; propodeum with apical carina complete or interrupted in middle; all abdominal segments basally black; hind tibia largely yellow or reddish brown 9

9. Occipital carina joining hypostomal carina at an acute angle, distance between junction of occipital and hypostomal carinae and base of mandible lesser than basal width of mandible 10

Occipital carina joining hypostomal carina at a right angle or broken; distance between junction of occipital and hypostomal carinae and base of mandible greater or lesser than basal width of mandible 14

10. Occipital carina interrupted dorsally; vertex polished and smooth behind; first four abdominal segments with complete yellow apical bands 11

Occipital carina complete dorsally; vertex punctate; abdominal segments with or without yellow bands apically 13

11. Basolateral area of propodeum rugoso-punctate; first four hind tarsal segments yellowish white, fifth fulvous; flagellum black with 7–14 segments with a yellowish white dorsal stripe ..... *L. (L.) philippinensis* Kamath

Basolateral area of propodeum reticulo-punctate; first and fifth hind tarsal segments brownish black, second and third light yellow; flagellum brownish black with 7–12 segments with a yellow dorsal stripe .... *L. (L.) acuminata* Gupta and Kamath..... 12

12. Flagellar segments 7–11 with a yellow dorsal stripe; fourth hind tarsal segment brownish black ..... *L. (L.) acuminata acuminata* Gupta and Kamath

Flagellar segments 8-12 with a yellow dorsal stripe; fourth hind tarsal segment yellowish white ..... *L. (L.) acuminata burmensis* Kamath

13. Pronotal collar without a tooth-like prominence on the lower margin; dorsal valve of ovipositor without median carina; vertex with distinct, fine, evenly distributed punctures ..... *L. (L.) keralensis* sp. nov.

Pronotal collar with a tooth-like prominence on the lower margin; dorsal valve of ovipositor with a median carina; vertex coarsely punctate, medially obliquely striated behind ..... *L. (L.) nigriabdominalis* Gupta and Kamath

14. Mandibular teeth equal; lower margin of pronotal collar without a tooth-like prominence 15

Mandibular teeth unequal; lower margin of pronotal collar with a tooth-like prominence 17

15. Occipital carina meeting hypostomal carina at about a right angle, not farther from base of mandible than basal width of mandible; occipital carina ventrally moderately incurved; postpetiole basally raised ..... (*L.*) *rugifrons* Cameron
    Occipital carina more or less interrupted at the junction with hypostomal carina, joining at more than a right angle, distance between junction of occipital carina and hypostomal carina and base of mandible greater than basal width of mandible; occipital carina ventrally sharply recurved; postpetiole evenly curved at base 16
16. Apical carina of propodeum complete in middle, sometimes weak; frontal horn 0.5*x* as long as scape; mesoscutum rugoso-punctate; hind tarsus infuscate to yellow with first, apical half of fourth and fifth segments black .....
    ..... *L. (L.) perfecta* Gupta and Kamath
    Apical carina of propodeum interrupted in middle, sometimes weak but broken; frontal horn 0.2–0.3*x* as long as scape; mesoscutum coarsely, deeply punctate; hind tarsus wholly brownish black ..... *L. (L.) confracta* Gupta and Kamath
17. Apical carina of propodeum incomplete, confined only around crests; hypostomal carina normal to moderately raised .....
    ..... *L. (L.) assamensis* Gupta and Kamath
    Apical carina of propodeum complete, but not stronger than other rugosities; hypostomal carina conspicuously raised 18
18. Distance between junction of occipital and hypostomal carinae and base of mandible equal to or less than basal width of mandible; occipital carina moderately curved ..... *L. (L.) armata* Cameron
    Distance between junction of occipital and hypostomal carinae and base of mandible greater than basal width of mandible; occipital carina sharply recurved 19
19. Basal portion of clypeus with scattered shallow punctures; apical yellow band on second abdominal segment absent except for stramineous spots on lateral and dorsolateral sides; second and third hind tarsal segments yellow .....
    ..... *L. (L.) coreensis chinensis* Kamath
    Basal portion of clypeus strongly punctured; apical yellow band on second abdominal segment present and complete; all hind tarsal segments dark brown, their subbases lighter in colour ..... *L. (L.) townesi* Kamath

Abbreviations

HL = Head Length; HW = Head Width; FWL = Fore Wing Length; FWW = Fore Wing Width; HWL = Hind Wing Length; HWW = Hind Wing Width.

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Haemolymph amino acids and protein profile in the tropical Tasar Silkworm, *Antheraea mylitta* (Drury) (Lepidoptera: Saturniidae)

D. D. Barsagade and D. B. Tembhare*

Department of Zoology, Nagpur University Campus, Nagpur 440010, India

ABSTRACT: Total haemolymph amino acid concentration declined initially since emergence to the sixth day old larvae and thereafter increased gradually till the onset of spinning. It again rapidly dropped down during the spinning period. The total haemolymph protein concentration initially increased since emergence upto the six day old larva, dropped down during the sixth to ninth day larval period and thereafter gradually increased prior to spinning. It declined during the spinning period. Total amino acid concentration in haemolymph thus showed parallel relationship with the secretory activity of the silkglands, suggesting their utilization in the synthesis of silk proteins besides in the development and growth of the last instar larva. Haemolymph proteins on the other hand did not show any correlation with the secretory phase of the silk glands and seems to be mainly used in growth and development of the larva. SDS-PAGE electrophoretic analysis of haemolymph proteins revealed separation of 10–15 protein bands among which some of them represent the storage and sex-specific proteins.

KEYWORDS: *Antheraea mylitta*, Haemolymph, amino acid, protein

INTRODUCTION

Various biochemical studies revealed periodic changes in the total concentration of haemolymph amino acids and proteins during metamorphosis in insects (Gilmour, 1965; Chen, 1971; Rockstein, 1978; Kilby, 1965; Levenbook, 1985). In the silkworm, *Bombyx mori* rapid uptake of at least some amino acids from haemolymph to the silk gland cells is reported (Amanieu *et al.*, 1965; Prudhomme and Chavancy, 1969; Prudhomme and Couble, 1979). Levenbook (1985) concluded from his pioneering studies on *B. Mori*, that the larval haemolymph proteins differ from the silk proteins that represent the storage proteins predominantly.

Biochemical studies on the tropical tasar silkworm, *Antheraea mylitta* are, however, very scanty (Agarwal *et al.*, 1974; Jolly *et al.*, 1979) and the present investigation was, therefore, undertaken to explore the haemolymph amino acids and protein profile.

*Corresponding author

MATERIAL AND METHODS

Haemolymph was collected by pricking the prolegs of newly emerged, 3, 6, 9, 12, 15, 18 and 21 day old fifth instar larvae of trivoltine tropical tasar silkworm, *Antheraea mylitta* in small vials, precoated with phenyl-thiourea to prevent melanization. The haemolymph samples were centrifuged immediately at 4500 rpm and the supernatant was stored at −20 °C till the total concentration of amino acids and protein estimation was carried out with the method of Moor and Stein (1954); Lowery *et al.* (1951) respectively. SDS-PAGE haemolymph protein was carried out by the method of Laemmli (1970) with some minor modifications (Barsagade, 1998).

The 1 mm 3% stacking gel (pH 6.8) was followed by a 10 ml 10% separating gel (pH 8.8) with 1% SDS 50 μl of clear supernatant was mixed with 50 μl of treatment buffer (Tris – 2.5 ml pH 6.8, SDS – 4 ml, Glycerol – 2 ml, 2-Mercaptoethanol – 1 ml, Distilled water – .5 ml and a pinch of Bromophenol blue). The samples were heated for 5 minutes in a water bath. The mixtures was cooled and its 25 μl, 30 μl, 35 μl quantity was separately applied onto the top of the gel. Standard broad range molecular weight marker protein was also run together. The gel was stained with Coomassie brilliant blue for 2 hours and destained with a mixture of methanol- acetic acid- distilled water until the bands on the gel became clear. The molecular weight of the protein bands with regard to the marker proteins were estimated with the help of the Densitometer.

RESULTS

Total haemolymph amino acid concentration

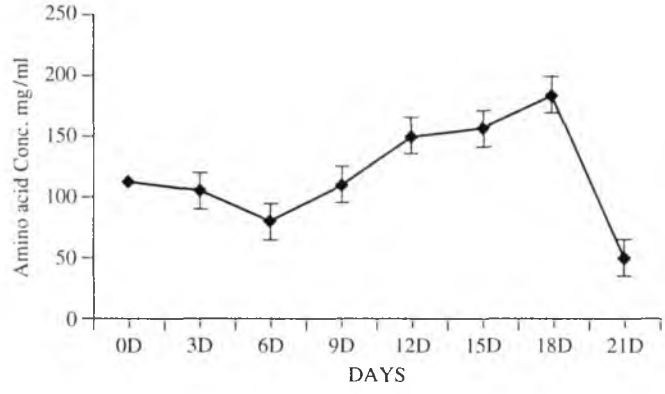
The total haemolymph amino acid concentration in newly moulted silkworm is about 112.5 ± 0.161 mg/ml. It decreased to 105.0 ± 0.23 and 80.5 ± 0.19 mg/ml on the third and sixth day, respectively. The total haemolymph amino acid concentration increased gradually upto 110.00 ± 0.145, 150.5 ± 0.31 and 157.0 ± 0.26 mg/ml on the 9th, 12th, and 15th day respectively and attained the maximum of 185.00 ± 0.28 mg/ml on the 18th day. Later on, it dropped down to 52.5 ± 0.13 mg/ml on the 21st day (Fig. 1).

Total haemolymph protein concentration

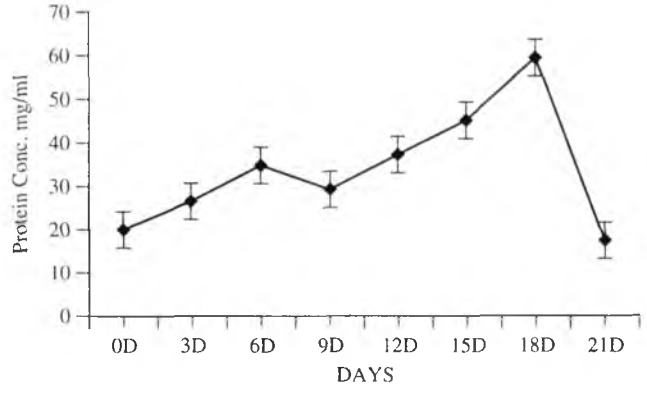
The total haemolymph protein concentration in newly moulted silkworm is about 20.10 ± 0.12 mg/ml. It increased to 26.5 ± 0.25 mg/ml on the third day and 35.00 ± 0.19 mg/ml on the sixth day but decreased to 29.5 ± 0.12 mg/ml on the ninth day. Total haemolymph protein concentration thereafter, increased gradually upto 37.5 ± 0.18 and 45.00 ± 0.31 on the 12th and 15th day, respectively and attained the maximum concentration of 60.00 ± 00.43 mg/ml on the 18th day. Later on, it dropped down to 18.00 ± 0.28 mg/ml on the 21st day (Fig. 2).

Eletrophoretic analysis of haemolymph proteins

The plasma proteins during larval development of *A. mylitta* have been analysed by 10% SDS Polyacrylamide Gel Electrophoresis (PAGE). Haemolymph of early fifth



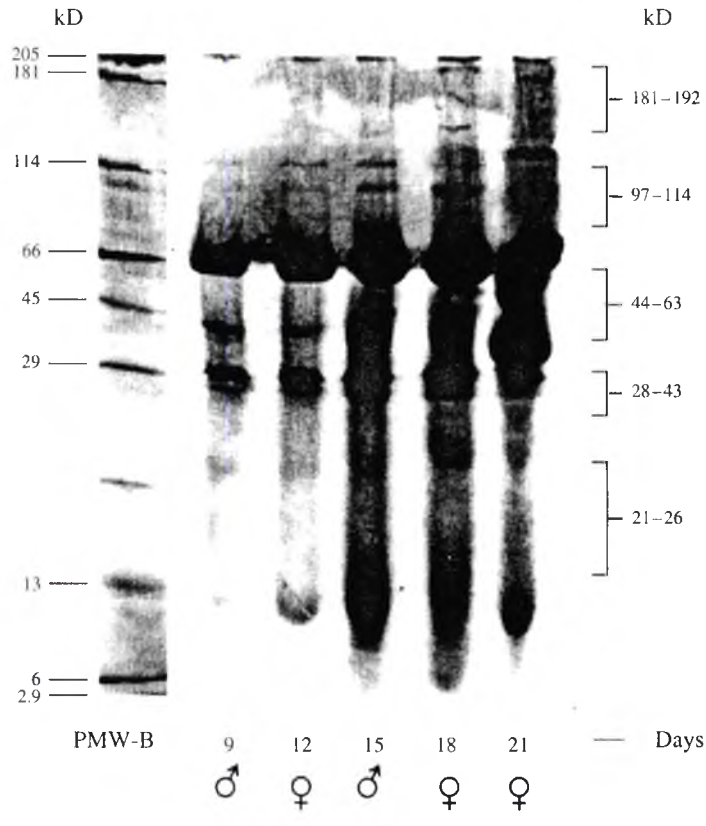
FIGURES 1. Haemolymph total amino acid concentration in the fifth instar larva since emergence till entering pupation



FIGURES 2. Haemolymph total protein concentration in the fifth instar larva since emergence till entering pupation

instar larvae (6 day old larvae) show approximately 12 cammassie blue-stained bands. In the mid fifth instar larvae, (12–day old larvae) two protein bands disappear and only 10 bands appear predominantly, while in the late fifth instar (18 day old larva) 15 dark bands are observed. During larval development, the plasma protein profile seems to change rapidly. The intensity of some protein bands increases towards the final day of larval development, the storage proteins with the molecular weight of 38–63 kD range can be divided into two groups as SP-1 and SP-2 respectively. The SP-1 shows a single but thick subunit of 63 KD, while SP-2 shows two thin subunits. The SP-1 and one of the SP-2 i.e. 38 kD appear since the first day and are predominant till the last day, while 45 kD SP-2 appears on the 15th day and both are predominant on the 18th day i.e. just before the larvae start the process of spinning (Fig. 3).

The plasma protein profile of *A. mylitta* shows another group of proteins, the 21–28 kD duplet band of proteins the 3 to 18 day old male and female larvae. Another



FIGURES 3. SDS-PAGE of haemolymph of 9–21 day old male and female fifth instar larvae

group of proteins of higher molecular weight is well evident in the 18 day old larvae. This 181–192 kD protein is found in the female larvae only representing the female sex proteins. The 97–114 kD protein is, moreover found in both the male and female larvae.

DISCUSSION

It is evident from the studies of Amanieu *et al.* (1965) on *B. mori,* that most of the amino acids, particularly glycine, tyrosine and serine are transported directly to the silk gland from the haemolymph, while alanine and tyrosine are synthesized in the silk gland cells which constitute major composition of fibroin. The amino acid uptake from the haemolymph during the synthesis of fibroin in the PSG is, however, a very rapid process (Prudhomme and Couble, 1979; Prudhomme and Chavancy, 1969). The present study indicates that some haemolymph amino acids are probably incorporated into fibroin in *A. mylitta* in the similar way as reported in *B. mori* although

direct evidence is at present, lacking. The haemolymph protein concentration increases gradually upto 6th day, drops down during a period from 6th to 9th day and again rises gradually from 9th to 18th day and lastly diminishes rapidly from 18th to 21st day in the last instar larvae of *A. mylitta*. This indicates that initially during the growth phase, haemolymph proteins are accumulated in the haemolymph and subsequently, used in the growth of the larvae. The second cycle of accumulation in the haemolymph occurs during a period of 9th to 18th day (secretory phase) at the maximal level and diminishes thereafter during the regression phase to bring about the transformation of larva into the pupa, representing storage proteins.

In the last instar larvae of *A. mylitta*, the haemolymph protein concentration does not co-relate with that of MSG and PSG protein concentration rising gradually during the secretory phase of the silk gland. While, the haemolymph amino acid concentration is inversely co-related with the silk gland protein concentration during secretory phase (Barsagade and Tembhare, 2000) suggesting consumption of amino acids in the synthesis of protein during initial period of first six days and thereafter their absorption into the silk gland and lastly, gradual accumulation of amino acids into the haemolymph forming a amino acid pool (Gilmour, 1965; Kilby, 1965).

There are several studies on the haemolymph protein concentration in *Bombyx. mori* suggesting their role in growth or metamorphosis of the larvae rather than contributing in silk protein synthesis (Levenbook, 1985).

According to (Roberts and Brook, 1989), 'the storage proteins are few in number and occur only in the larval stages where they accumulate in the haemolymph. They are synthesized predominantly by the larval fat body and their concentration increases enormously in the last larval instar'. All storage proteins have a molecular weight in the range of 50,000 and are composed of six subunits and are also called as the larval haemolymph proteins after denoted as LHP-1, 2 etc. (Levenbook, 1985). Among the total plasma proteins, the SP-1 and SP-2 become major fat body constituents among which, the SP-1 is female specific and SP-2 is a simple haemolymph protein (Rockstein, 1978). In *A. mylitta* although fifteen protein bands occur intensly in the late fifth instar larvae, the male and female larvae show sexual dimorphism. The presence of SP-1 and SP-2 in *A. mylitta* during larval development is similar to that in *B. mori* (Tojo *et al.*, 1980; Mine *et al.*, 1983) and the SP-1 can be considered as a vitellogenin in *A. mylitta*.

Although 30 kD proteins were reported in *Bombyx mandarina* (Sakai *et al.*, 1988) and *B. mori* (Mahalingam, 1991) they have not yet been reported in other lepidopteran species. The 21–26 kD proteins appeared from third day of development till the time of spinning in *A. mylitta*. The peculiar feature of these proteins is the composition of triplet bands at the late larval stage representing the delayed synthesis of these proteins (Mahalingam, 1991). The present studies, however, do not support the observations by (Mahalingam, 1991) that these proteins cover more area and are preferentially synthesized in the male than in the female as has been found in *B. mori*. Besides SP-1, another storage protein in *A. mylitta* is confined to only the female larva, which appears as the 181–192 kD protein band on the 18th day. It certainly seems to be

another female specific protein, i.e. vitellogenin similar to that reported in *B. mori* (Mahalingam, 1991).

It is now well established that the larval haemolymph proteins of *B. mori* differ from the silk proteins not only in molecular weight, but also in their mRNAs and tRNAs, as well as in their genes (Levenbook, 1985) and It might be true in *A. mylitta* also since these proteins appear independently in the electrophoretic preparations.

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New species of carpenterbee (Hymenoptera: Apidae: Xylocopinae) recorded from Northwest India

Avdhesh Kumar* and Alexander Lall

School of Entomology, St. John's College, Agra 205002, India

ABSTRACT: A new species *Xylocopa (Koptortosoma) sanctijohani* sp. nov. under subfamily *Xylocopinae* is described from Northwest India based on male genitalia.

KEYWORDS: N. W. India, *Xylocopa*, new species, *sanctijohani*

INTRODUCTION

Bingham (1897) treated the family Xylocopidae under the Heterogenous assemblage of family Apidae. He treated 18 carpenter bee species under a single genus *Xylocopa* Latr. Ashmead (1899) divided genus *Xylocopa* into five genera. Gupta (1960) studied some hymenopterans collected by Prof. M. S. Mani's third Entomological Expedition to the Northwest Himalaya. He recorded one species of *Xylocopa* for the first time from the higher reaches of Kullu and Lahaul Spiti Valleys. We are indebted to Ma, Tsing, Chao (1938), who studied the Indian species of *Xylocopa*. In his studies he erected some new subgenera like, *Biluna, Ctenopoda, Nodula, Zonohirsuta* and *Orbitella*. Minckley (1998) did cladistic studies on the subgenera of carpenterbees. We have followed the classification of Apidae given by Michener (2000), who treated family Xylocopidae as subfamily Xylocopinae of Apidae.

MATERIALS AND METHODS

The first author collected the specimens using sweep net at the time of foraging from different parts of Northwest India during 1992–1998. Specimens were pinned and stored in wooden boxes with Para dichlorobenzene. The mouth parts, male genitalia, antennae and legs were treated with 10% KOH solution for about two days at 35 °C room temperature and after dehydration mounted in DPX.

*Present address: Department of Entomology, College of Agriculture GBPUA&T, Pantnagar, India

RESULTS AND DISCUSSION

Xylocopa (Korptortosoma) sanctijohani sp. nov. Male (Plate I, A–F)

Head

Ocelli three, arranged in a triangle, all ocelli similar in size, upper two ocelli present in a raised portion of the vertex, third ocellus present in a depression on the frons, upper ocelli separated by two and a half diameters from eyes. Antennal segments 3 : 4 : 5 : 6 = 6 : $2\frac{1}{2}$: 3 : $3\frac{1}{2}$, third segment about three times as long as broad. Inner orbits curved, weakly divergent at their upper extremities. Frontal keel weak. Apical margin of supra clypeal region and basal margin of clypeus distinctly elevated, clypeus convex, medially unpunctated, transverse ridge of labrum weak. Mandibles bidentate, outer proximally large tooth pointed apically, inner tooth very short, blunt, rounded, outer margin with long bristles basally and short bristles apically. Ocellocular distance 0.60 mm, interocellar distance 0.80 mm and ratio of ocellocular: interocellar 3 : 4. Antennocular distance 0.72 mm, interorbital distance 2.88 mm and ratio of antennocular: interorbital distance 1 : 4. Verticoclypeus distance 3.68 mm, more than five times as long as antennocular distance. Maxillary palpi six segmented, apically five decreasing in length and thickness successively. Proboscis elongated, pubescent, 5.24 mm long. Labial palpi four segmented, basal two segments minute, labial palpi 2.8 mm long, first segment of labial palpi nearly four times as long as second segment.

Thorax

Thorax deeply punctuated, middle scutal furrow reaching almost middle of scutum, lateral scutal furrow clear. Fore, middle and hind legs punctated, pubescent; metatarsi and tarsi with dense, pubescence, knee caps simple, inner and outer teeth of claws weakly divergent. Wings transparent, subhyaline, 14.32 mm long, length of marginal cell 4.8 mm.

Abdomen

Abdomen with 7 visible tergites, all tergites dark pubescent, lateral margin of tergites and sternites pubescent. Epipygium medially furrowed, posteriorly with submedial processes. Hypopygium apically curved, medial keel prominent.

Male Genitalia (Plate I, F)

Cardo rounded basally. Outer margins of stipes convex, inner margin curved, Squama globular, setae long marginally. Lacinia fused to squama. Sagitta broad basally, narrow apically, distally with leaf like processes, few setae in middle of outer margin curved towards squama. Spatha broad.

Colouration

Integument reddish black, body covered dorsally with yellow pubescence, eyes and supraclypeal region with scattered yellow pubescence, clypeus without pubescence.

PLATE

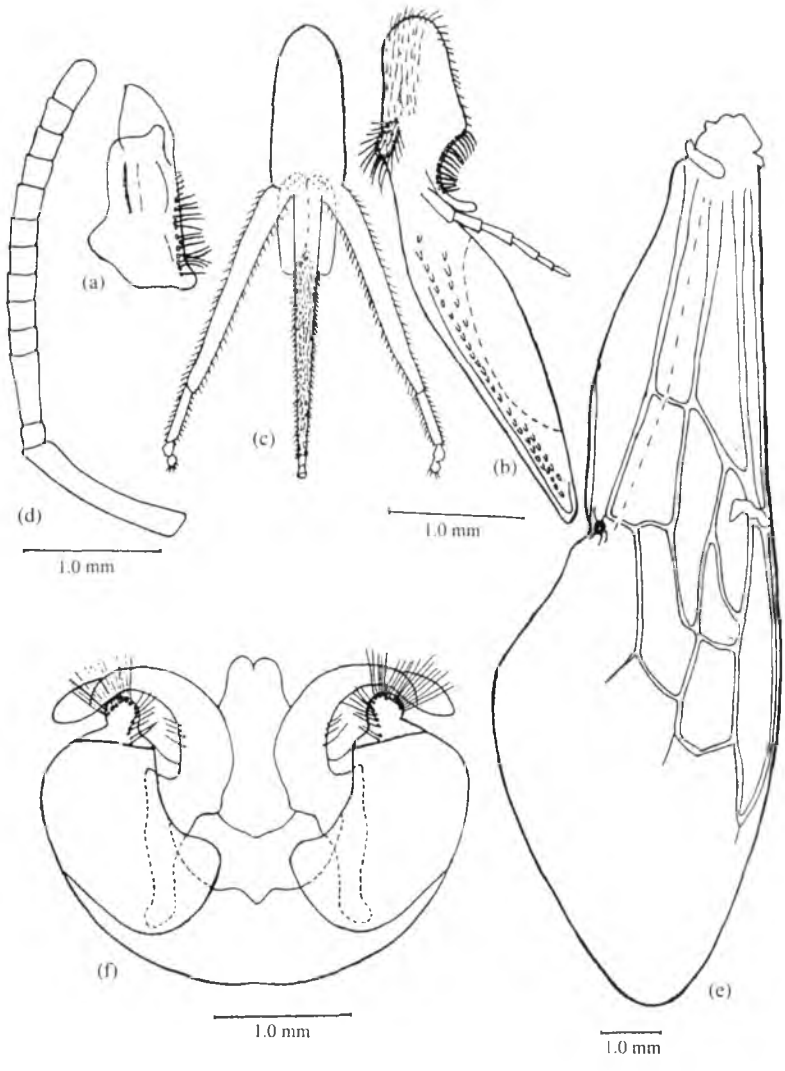

PLATE I: Plates of *Xylocopa (Orbitella) sanctijohani* sp. nov. Fig. A. Mandible, B. Labium, C. Proboscis with labrum, D. Antenna, E. Fore wing, F. Male genitalia

Frons, vertex, temple and gena covered with dense yellow pubescence. Thorax globose with deeply shining yellow pubescence dorsally. Sides of thorax, base of wings and tegula more pubescent. Thoracic sternites with blackish pubescence, lateroventrally with yellow pubescence, outer margin of legs covered with light yellowish pubescence, inner margin covered with yellow pubescence. Abdominal segment 1–6 with yellow pubescence, 7th tergite with blackish pubescence laterally and yellow pubescence medially, venterolateral sides of tergites and sternites with

yellowish white pubescence, 6th sternite with black pubescence, median portion of 1–5 sternites reddish and smooth.

Size

Body length of Male 22-2mm; Female not recorded.

Type

Holotype

Male, Agra (U.P.) Coll. Avdhesh Kumar, 14. IV.1994. (Mouth parts, wings, legs and male genitalia on slides)

Paratype

1♂, Agra (U.P.) Coll. Avdhesh Kumar, 14.IV. 1994; 2♂♂, Haridwar (U.P.) Coll. Avdhesh Kumar 26.IV.1995; 1♂ Jaipur (Raj.) Coll. Avdhesh Kumar 10.V.1996.

Affinities

Xylocopa (Kortortosoma) sanctijahani sp. nov. comes close to *Xylocopa verticalis* (Lepeletier) but it differs markedly in the structure of male genital armature. Besides, it has globose and setaceous squama and flap like sagitta compared to elongate squama and elongate and terminally blunt, sagitta in *Xylocopa verticalis*.

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Effect of extract of the seaweed *Bryopsis plumosa* (Huds.) Ag. on the feeding rate and protein profile of haemolymph and fat body of *Hyblaea puera* (Cramer) (Lepidoptera: Hyblaeidae)

B. Biju[1], Mariamma Jacob[1], K. Padmakumar[2] and D. Muraleedharan*[1]

[1]*Department of Zoology, University of Kerala, Kariavattom, PO, Trivandrum, Kerala*
[2]*Department of Aquatic Biology and Fisheries, University of Kerala, Kariavattom PO, Trivandrum, India*

ABSTRACT: Ethanol extract of the seaweed, *Bryopsis plumosa* was screened for antifeedant property against the teak defoliator, *Hyblaea puera*. Oral treatment of *B. plumosa* extract at a dosage of 50 μg and upto 200 μg showed reduced leaf consumption. Larval mortality was maximum at higher dose of 200 μg. Haemolymph and fat body of treated and control larvae were subjected to electrophoresis (SDS PAGE) and significant changes in protein pattern were observed after treatment of the extract. Proteins in the haemolymph and fat body showed significant reduction.

KEYWORDS: *Hyblaea puera*, *Bryopsis plumosa*, antifeedant, protein profile

INTRODUCTION

Antifeedants are substances which can inhibit or reduce feeding rate of animals and thereby affect all systems of the body. Most of the prevalent antifeedants are of plant origin. Antifeedants obtained from plants are mostly ecofriendly and hence safe for controlling insects. Diverse secondary metabolites present in many types of seaweed have defensive action against certain fishes and invertebrates (Hay and Fenical, 1988; Hay *et al.*, 1990; Padmakumar and Lali, 1997; Paul and Van Alstyne, 1988). *Bryopsis plumosa*, a seaweed belonging to Class Chlorophyceae was found to possess such antifeedant activity against *Sylepta derogata* (Bindu and Muraleedharan, 2001) and some natural fish predators (Becerro *et al.*, 2001). Seaweed extracts may offer a novel approach to pest management.

*Corresponding author

In the present study, the possible antifeedant activity of extracts of *B. plumosa* on larvae of *Hyblaea puera* (Cramer) (Lepidoptera: Hyblaeidae), a serious defoliator pest of teak, *Tectona grandis* (Beeson, 1941; Nair *et al.*, 1985) was investigated. Changes induced by the extract on haemolymph and fat body proteins of *H. puera* larvae were studied.

The alga *Bryopsis plumosa* was collected at Rameswaram (9°15′N; 79°15′E) from the intertidal region to a depth of 1 m during December, 1995. Immediately after collection, the alga was washed in sea water and the epiphytes, associated organisms and other extraneous matter were removed. After subsequent washing in fresh water, the plants were air-dried at shade and then in an incubater at 40 ± 2° C. The dried material was powdered to fineness using a mechanical blender. About 250 gm of finely powdered plant material was soaked in 500 ml of 80% ethanol at room temperature for 24 h. The resultant extract was concentrated to dryness under reduced pressure at 40 ± 2° C. The crude extract obtained was kept frozen until the antifeedant assay (Padmakumar, 1998).

Fourth instar larvae of *H. puera* were used for the study. Larvae collected from teak plantations at Kulathupuzha, near Trivandrum were reared in the laboratory at a temperature 27 ± 3° C; relative humidity 85 ± 5% and photoperiod 12 : 12 light and dark regime. To test the feeding rate and protein profile with *B. plumosa* extract, it was applied topically and orally. For topical application, the crude extract was dissolved in ethanol (50 mg in 10 ml) and doses of 50, 100, 150 and 200 μg were applied on the dorsal side of pre-starved fourth instar larvae.The treated larvae were allowed to feed. The feeding rate of treated larvae was compared with that of larvae treated with ethanol alone.

For oral application, different concentrations of the extract 50, 100, 150 and 200 μg, were applied to leaf discs, 0.7 cm^2, cut out from fresh leaves and provided to pre - starved larvae. Control group was fed with leaf discs treated with ethanol only. After one hour the two sets of larvae were allowed normal food. The difference in leaf area consumption was estimated by plotting partially consumed leaves on graph paper.

Electrophoresis on SDS-PAGE was carried out with haemolymph and fat body samples by the method of Hames (1987) with minor modifications. Haemolymph samples were collected by cutting the pro-leg of the larva. The exuded haemolymph was collected by a micropipette into a pre-chilled eppendorf tube containing a few crystals of phenyl thiourea (PTU). The supernatant was mixed with double volume of sample buffer (0.5 M Tris-HCl, pH 6.8, 10% SDS, β-mercaptoethanol, glycerol) and incubated for 3 min. Then the sample was centrifuged for 10 min. at 10,000 rpm. The supernatant was used as loading sample.

Fat body sample was collected, homogenized in 0.5 M Tris-HCl (pH 6.8) and centrifuged for 10 minutes at 10,000 rpm. The supernatant was transferred into an eppendorf tube containing an equal volume of sample buffer (0.5 MTris-HCl, pH 6.8, 10% SDS, β-mercaptoethanol, glycerol).Then the sample was incubated for 3 min. The heated sample was centrifuged for 5 min. at 10,000 rpm. The supernatant was used as the loading sample. Both of the experiments were repeated five times.

TABLE 1. Effect of oral and topical application of *B. plumosa* extract on feeding rate of fourth instar *H. puera* larvae

Concentration of algal extract μg	Leaf area consumed cm^2 (Mean $\pm$ SD)					
	Topical application			Oral Application		
	Control	Treated	% reduction	Control	Treated	% reduction
50	496.4 $\pm$ 8.2	441.4 $\pm$ 1.6	11.1	491.6 $\pm$ 11.0	315.8 $\pm$ 5.6	35.8
100	492.4 $\pm$ 8.0	421.3 $\pm$ 5.3	14.4	499.2 $\pm$ 1.4	285.2 $\pm$ 5.7	42.9
150	497.3 $\pm$ 6.5	386.6 $\pm$ 4.1	22.3	498.2 $\pm$ 7.9	241.6 $\pm$ 9.0	51.6
200	500.4 $\pm$ 8.0	330.4 $\pm$ 5.2	34.0	500.2 $\pm$ 5.1	192.8 $\pm$ 7.4	61.6

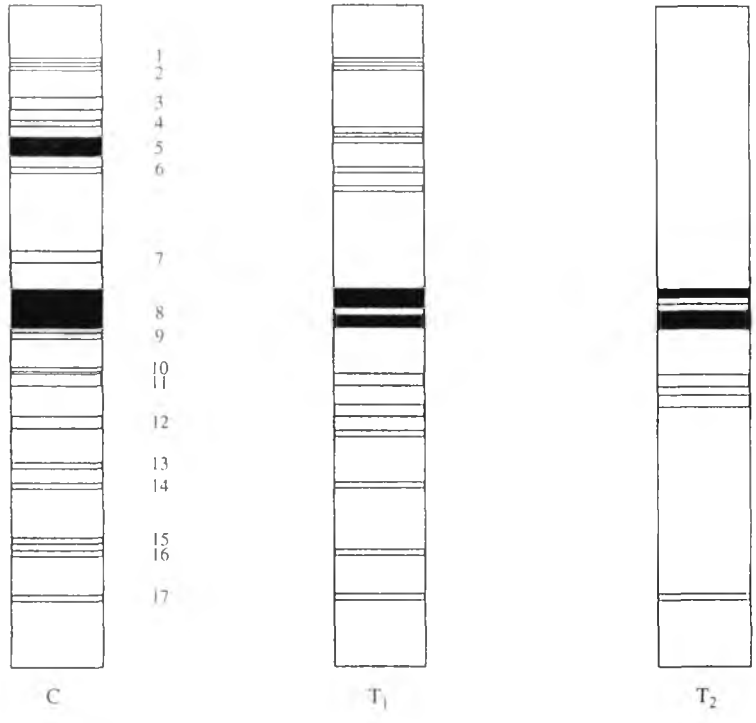
Results are expressed as Mean $\pm$ SD of five samples ($n = 5$).
% reduction in feeding over controls

Data on the effect of *B. plumosa* extract on feeding rate of *H. puera* larvae are given in Table 1. Both topical and oral administration of the extract inhibited feeding activity. Oral application was more effective causing 35.8 to 61.6% reduction in feeding activity at 50 to 200 μg dose. The effect on feeding was dose dependent and most of the low dose treated ones resulted in reduction in larval growth and moulted with wing deformities.

There was considerable difference on the electrophorogram of haemolymph and fat body protein profile of control and treated groups of orally treated larvae. In the haemolymph sample of control (C-Fig. 1), 17 bands were present, 5 and 7th bands were thick and most prominent, highly darkened compared to other bands. In 100 μg treated larvae the thick 5th band was completely absent, 7th band was also absent and 8th band was darkened but not as thick as that of control. In addition, 12th band was feeble (T1 – Fig. 1). In 200 μg treated larval sample, only five bands were seen which indicated further reduction of proteins as the dosage increased. This showed that *B. plumosa* extract reduced quantity and quality of the protein profile of haemolymph in *H. puera.*

In the fat body protein samples of orally treated larvae (Fig. 2), there were 18 bands in control, 4th band was more broadened and darkened (Co). In 50 μg-treated larvae, there was considerable reduction in the number of protein bands (t4). In the 100 μg-treated larvae (t_3) and in 150 μg treated larvae (t_2) there was still reduction in the number of protein bands compared to that of control. In 200 μg treated larvae (t_1) only five bands were seen. These results were clear evidences to prove that *B. plumosa* extract had affected feeding and which in turn had affected the normal physiology of the insect and it was dose dependent.

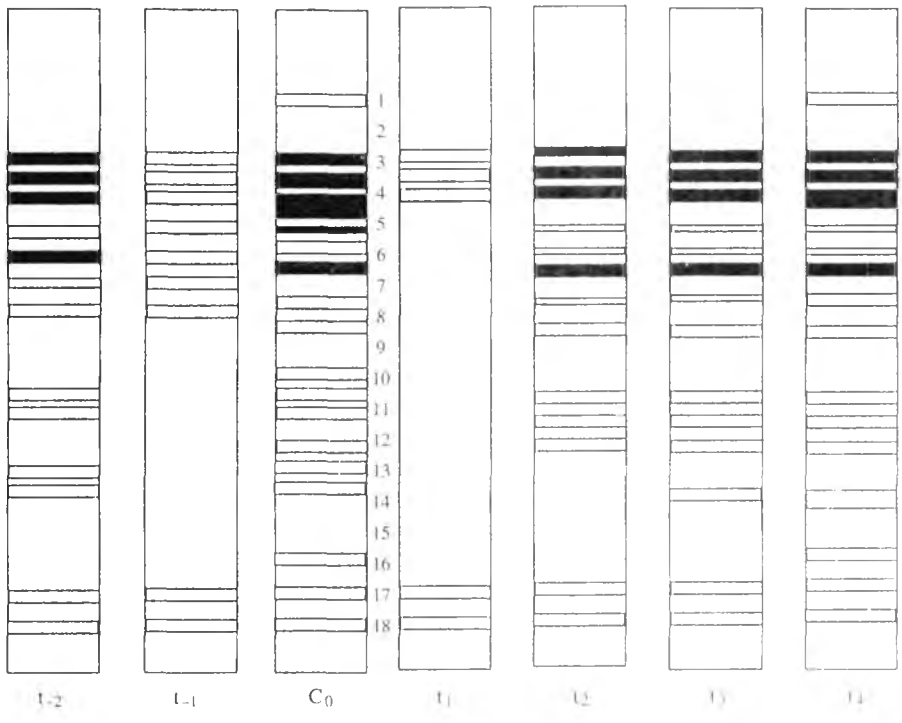
Significant difference was also observed in the protein bands in the fat body of topically treated larvae. Only two concentrations (100 and 200 μg) were used for this study. As described above, there was marked reduction of protein bands. Only thirteen



FIGURES 1. Diagramtic representation of electrophoretic protein pattern of haemolymph sample in treated and control *H. puera*.
C – control; T_1 – *B. plumosa* extract treated (100 μg), T_2 – *B. plumosa* extract treated (200 μg)

bands were detected in 100 μg treated larvae (t_{-2}) and in 200 μg treated larvae, about 9 bands which were feeble in appearance could be counted (t_{-1}). From this it is evident that, the algal extract affected the protein profile in the fat body of *H. puera*. As the dose of the extract increased, proteins of the haemolymph and fat body were reduced.

The present study reveals that *B. plumosa* extract reduced feeding rate of *H. puera* and subsequently due to starvation the protein profile in haemolymph and fat body was reduced. It was observed that *B. plumosa* extract had a dose dependent inhibition on larval growth and produced wing deformities in *H. puera*. There are a number of reports on the antifeedant activity of plant extracts. Kulkarni *et al.* (1996a) reported the antifeedant activity of *Lantana camara* and *Aloe vera* leaves on another pest of teak *Eutectona machaeralis*, and Kulkarni and Joshi (1997) reported insecticidal activity of some plant extract on Albizia defoliator. Murugesan *et al.* (1998) had found the biopesticidal activity of neem products against forest tree species. However the antifeedant activity of seaweed extract is comparatively a new introduction in biopesticide research. El Sayed *et al.* (1998) reported the marine organisms as a



FIGURES 2. Diagramatic representation of electrophoretic protein pattern of fat body sample in treated and control *H. puera*.
t_2 – 150 μg of extract topically applied; t_1 – 200 μg of extract topically applied; C_0 – Control; t_1 – 200 μg of extract orally applied; t_2 – 150 μg of extract orally applied, t_3 – 100 μg of extract orally applied; t_4 – 50 μg of extract orally applied.

rich reservoir of biologically active natural products, especially as lead molecules for the development of new drugs and insecticides. Hay *et al.* (1990) had found that secondary metabilites present in many types of seaweeds have defensive action against certain fishes and invertebrates. According to Becerro *et al.* (2001) the mollusk *Elysia rufescens* develop chemical defence due to its host alga *Bryopsis* sp. In the present study on *H. puera*, the protein profile of haemolymph and fat body was considerably reduced due to the antifeedant activity of *B. plumosa*. Bindu and Muraleedharan (2001) reported that *Bryopsis plumosa* had reduced feeding and protein profile in cotton leaf roller *Sylepta derogata* Fabr. Similar studies on *Bryopsis plumosa* are not available currently, but a number of studies on plant extracts showed that proteins present on fat body and haemolymph was reduced due to antifeedant activity (Padmaja and Rao, 2000; Jadhav and Ghule, 2003). The present study is only a preliminary step as it is a laboratory assessment and further studies are to be carried out to confirm the pesticidal quality of *B. plumosa* for controlling insect pests.

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New record of *Cydia ptychora* Meyrick (Tortricidae: Lepidoptera) on *Cajanus cajan* in Manipur

Sinam Subharani and T. K. Singh*

Department of Life Sciences, Manipur University, Canchipur 795003, Imphal, Manipur, India

ABSTRACT: The pod borer, *Cydia ptychora* (Tortricidae: Lepidoptera) was recorded for the first time as pest of *Cajanus cajan* from Manipur.

KEYWORDS: *Cajanus cajan*, *Cydia ptychora*, new record, Manipur

Pigeonpea *Cajanus cajan* (Linn.) Millsp. commonly known as red gram or arhar is one of the important pulse crops and is known to harbour many pod borer species during the reproductive phase. The pod borers are a major pest complex, damaging the flower buds, flowers, pods and seeds. So far, 27 species of pod borers are recorded on pigeonpea in India (Chaudhury and Bhattacharya, 1974, Lal *et al.*, 1985). Survey conducted in India has shown pod damage varied from 33.8% in North zone to 49.9% in South zone with the national average of 43.9%. (Bhatnagar *et al.*, 1982; Lateef and Reed, 1983).

While studying the population dynamic of the pod borer complex of pigeonpea in Manipur, *Cydia ptychora* was found infesting the crop. The infestation by *C. ptychora* was observed during the flowering stage and continued till the pod maturing. Various workers (Ram *et al.*, 1981; Shantibala *et al.*, 2001; Devi and Singh, 2001a; Devi *et al.*, 2002) had reported other pod borers *viz.*,. *Etiella zinckenella* (Triet), *Exelastis atomosa* (W.) *Catechrysops cnejus* Fb., *Lampides boeticus* (Linn.) *Apion ampulum* (Fst.), *Maruca testulalis* (Geyer), *Melanagromyza obtusa* (Malloch) from different pigeonpea growing areas of Manipur. This is the first report of *C. ptychora* as a pest of pigeonpea from Manipur.

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*Corresponding author

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Aleyrodid (Hemiptera: Aleyrodidae) fauna of the Lakshadweep, India

A. K. Dubey[1], K. Regu[2] and R. Sundararaj[*3]

[1]*Insect Museum, Centre for Ecological Sciences, Indian Institute of Science, Bangalore 560012, Karnataka, India*
Email: anil2kd@yahoo.com
[2]*Regional Filaria Training and Research Centre, Karaparamba, Calicut 673010, Kerala, India*
[3]*Institute of Wood Science and Technology, Malleswaram, Bangalore 560003, Karnataka, India*
Email: rsundar@iwst.res.in

ABSTRACT: A survey revealed the occurrence of 12 species of whiteflies representing 11 genera in Lakshadweep. They were found on plants representing 9 families. *Bemisia tabaci* (Gennadius) was found breeding on four host plants, *Dialeuropora decempuncta* (Quaintance & Baker) on three, and *Aleurodicus dispersus* Russell on two. The plant species *Thespesia populnea* (L.) Corr. harboured four species of whiteflies, viz., *Aleuroclava complex* Singh, *Aleurodicus dispersus* Russell, *Aleurolobus marlatti* (Quaintance) and *Bemisia tabaci* (Gennadius).

KEYWORDS: whiteflies, Lakshadweep

The family Aleyrodidae (Insecta: Hemiptera) includes insects which are commonly known as whiteflies. They have long been known as pests of agricultural and horticultural importance. In terms of number of described species world-wide, the Aleyrodidae is the smallest of the four groups of Sternorrhyncha with 1200 species (Martin, 1999). Though aleyrodids were recorded in India as early as 1895 (Maskell, 1895), it was only in 1976, David and Subramaniam gave a new inpetus to taxonomic studies of Indian Aleyrodidae. Following this, significant contributions were made (David, 1987; David and Jesudasan, 1988; Jesudasan and David, 1991; David and Sundararaj, 1993; Sundararaj and David, 1993; Regu and David, 1993). However, no information is available on the whitefly fauna of Lakshadweep (8° and 120–300′ North latitude) except for the report of Ramani (2000), on the occurrence of *Aleurodicus dispersus* Russell. There are in all 27 islands and a number of sunken banks, open reys and sand banks. Only 10 islands are inhabited while the other islands are small and

*Corresponding author

exist as satellites of the inhabited islands. Aleyrodids were collected from three of the 10 inhabited islands and the findings are presented in this communication.

The whitefly infested leaves were collected from the host plants and the best mounts were obtained from pupal cases from which adults have emerged. The best mounts of pupal cases were prepared as suggested by Martin (1987) and the whiteflies were identified and confirmed with the guidance of B. V. David.

A total of 72 specimens were mounted representing 12 species. The subfamily Aleurodicinae was represented by one species and Aleyrodinae was represented by 11 species.

Subfamily: Aleurodicinae Quaintance and Baker

1. *Aleurodicus dispersus* Russell

Aleurodicus dispersus Russell, 1965. *Florida Entomol.*, **48**: 49–54.

Specimens examined

4 pupal cases on slides, on *Thespesia populnea* (L.) Corr, Androth (Lakshadweep), 27.iv.1997, Coll: K. Regu; 2 pupal cases, on *Tecoma stans* (L.) Kunth, Androth (Lakshadweep), 28.iv.1997, Coll: K. Regu.

Hosts

481 host plants in the world and 253 host plants from India (Srinivasa, 2000).

Distribution

Widely distributed in India (Srinivasa, 2000).

Subfamily: Aleyrodinae Westwood

2. *Aleurocanthus* sp.

Aleurocanthus Quaintance and Baker, 1914. *US Department of Agriculture, Technical Series, Bur Entomol.*, **27**: 102.

Type species

Aleurocanthus spinifera Quaintance, 1903. 61; by original designation.

Specimens examined

4 pupal cases on slides, on *Citrus* sp., Androth, (Lakshadweep), 28.iv.1997, Coll: K. Regu.

Host

Citrus sp.

Distribution

India: Lakshadweep (new distribution record).

3. *Aleuroclava complex* Singh

Aleuroclava complex Singh, 1931. *Mem. Dep. Agric. India (Ent. Ser.)*, **12(2)**: 91.

Aleuroclava complex Singh: Sundararaj and David, 1993. *Oriental Ins.*, **27**: 233-239.

Specimens examined

3 pupal cases on slides, on *Thespesia populnea* (L.) Corr., Androth (Lakshadweep), 27.iv.1997, Coll: K. Regu; 7 pupal cases, on *Lawsonia inermis* L., Androth (Lakshadweep), 28.iv.1997, Coll: K. Regu; 3 pupal cases, on *Ficus racemosa* L., Androth (Lakshadweep), 28.iv.1997, Coll: K. Regu.

Hosts

Three host plants *viz.*, *Ficus religiosa* L., *Aegle marmelos* Correa, *Madhuca latifolia* (Roxb.) McBride were listed as hosts in India (Sundararaj and David, 1993); *Lawsania inermis* L., *Ficus racemosa* L., *Thespesia populnea* (L.) Corr. (new host records).

Distribution

India: Bihar, Andhra Pradesh, Tamil Nadu (Sundararaj and David, 1993); Lakshadweep (new distribution record).

4. *Aleurolobus marlatti* Quaintance

Aleurodes marlatti Quaintance, 1903. *Can. Ent.*, **35**: 61–63; by original designation.

Aleurolobus niloticus Priesner and Hosny: Martin, 1999. *CSIRO Entomology Technical Paper*, **38**: 43–44 (Synonymised by Martin, 1999).

Specimens examined

3 pupal cases on slides, on *Thespesia populnea* (L.) Corr., Androth (Lakshadweep), 28.iv.1997, Coll: K. Regu.

Hosts

Reported to breed on 41 host plants in India (Regu and David, 1993).

Distribution

Many states of India (Regu and David, 1993), Lakshadweep (new distribution record).

5. *Bemisia grossa* Singh

Bemisia grossa Singh, 1931. *Mem. Dep. Agric. India*, **12(1)**: 82.

Specimens examined

1 pupal case on slide, on *Derris* sp., Androth (Lakshadweep), 28.iv.1997, Coll: K. Regu; 10 pupal cases, on unidentified plant, Minicoy (Lakshadweep), 3.v.1997; Coll: K. Regu.

Hosts

Eugenia operculata Roxb. (Singh, 1931), *Derris* sp. (new host record).

Distribution

India: Bihar (Singh, 1931), Lakshadweep (new distribution record).

6. *Bemisia tabaci* (Gennadius)

Aleurodes tabaci Gennadius, 1889 *Ellenike Georgia* (Greek Agriculture) (Athens), **5**: 1–3.
Bemisia tabaci (Gennadius) Takahashi, 1936: 110

Specimens examined

4 pupal cases on slides, on *Thespesia populnea* (L.) Corr., Androth (Lakshadweep), 27.iv.1997, Coll: K. Regu; 5 pupal cases, on *Ficus racemosa* L., Androth (Lakshadweep), 28.iv.1997, Coll: K. Regu.

Hosts

Many host plants (Jesudasan and David, 1991)

Distribution

Widely distributed in India (Jesudasan and David, 1991) Lakshadweep (new distribution record).

7. *Dialeurodes citri* (Ashmead)

Aleyrodes citri Ashmead, 1885 *Florida Dispatch*, **2(42)**: 704.
Dialeurodes citri: Quaintance and Baker, 1916. *J. Agric. Res.*, **6**: 469.

Specimens examined

7 pupal cases on slides, on unidentified plant, Androth (Lakshadweep), 28.iv.1997, Coll: K. Regu.

Hosts

Jasminum sambac Ait., *Hiptage madablota* (L.) Kurz., J. *arborescence* Roxb., *Ricinus communis* L., *Ardisia humilis* Vahl., *Citrus* spp., *Syzygium jambos* (L.) Alston, and *Jasminum* sp. were listed as hosts in India (Jesudasan and David, 1991).

Distribution

India: Assam, Meghalaya, Bihar, Uttar Pradesh, Uttaranchal and Punjab (Jesudasan and David, 1991); Lakshadweep (new distribution record).

8. *Dialeuropora decempuncta* (Quaintance and Baker)

Dialeurodes (*Dialeuropora*) *decempuncta* Quaintance and Baker, 1917. *Proc. U.S. Natn. Mus.*, **51**: 434.
Dialeuropora decempuncta (Quaintance and Baker): Takahashi, 1934. *Rep. Dep. Agric. Govt. Res. Inst. Formosa*, **63**: 46.

Specimens examined

5 pupal cases on slides, on *Cassia* sp., Androth (Lakshadweep), 28.iv.1997, Coll: K. Regu; 3 pupal cases, on *Ficus racemosa* L., Androth (Lakshadweep), 28.iv.1997, Coll: K. Regu.

Hosts

David and Subramaniam (1976) listed 12 host plants in India.

Distribution

India: Bihar, Uttar Pradesh, Andhra Pradesh, Tamil Nadu (David and Subramaniam, 1976); Lakshadweep (new distribution record).

9. *Indoaleyrodes laos* (Takahashi)

Dialeurodes laos Takahashi, 1942. *Trans. Nat. Hist. Soc. Formosa*, **32**: 329.
Indoaleyrodes laos: Martin, 1985. *Bull. British Mus. Nat. Hist. (Ent.)*, **50(3)**: 327.

Specimens examined

4 pupal cases on slides, on *Terminalia catappa*, Minicoy (Lakshadweep), 3.v.1997, Coll: K. Regu.

Hosts

Morinda tinctoria Roxb. (David and Subramaniam, 1976), *Terminalia catappa* L. (new host record).

Distribution

India: Tamil Nadu (David and Subramaniam, 1976), Lakshadweep (new distribution record).

10. *Kanakarajiella vulgaris* (Singh)

Dialeurodes vulgaris Singh, 1931. *Mem. Rep. Dept. Agric. India, Ent. Ser.*, **12(1)**: 33–34.
Kanakarajiella vulgaris (Singh): David and Sundararaj, 1993. *J. Ent. Res.*, **17(4)**: 233.
This species is reported as pest on *Jasminum* spp. in India.

Specimens examined

4 pupal cases on slides, on unidentified plant, Androth (Lakshadweep), 28.iv.1997, Coll: K. Regu.

Hosts

Nine host plants are listed from India (Meganathan and David, 1994).

Distribution

India: Bihar, Karnataka, Kerala, Tamil Nadu (Meganathan and David, 1994); Lakshadweep (new distribution record).

11. *Massilieurodes homonoiae* (Regu and David)

Dialeurodes (*Gigaleurodes*) *splendens* Regu and David, 1992. *J. Bombay Nat. Hist. Soc.*, **89**: 82–87.
Massilieurodes (*Gigaleurodes*) *homonoiae* (Regu and David) [Synonymised by Sundararaj and David], 2004. *Entomon*, **28(4)**: 371–372

Specimens examined

10 pupal cases on slides, on unidentified plant, Minicoy (Lakshadweep), 3.v.1997, Coll: K. Regu.

Host

Homonoia riparia Lour.

Distribution

India: Tamil Nadu (Regu and David, 1992), Lakshadweep (new distribution record).

12. *Pealius spina* (Singh)

Dialeurodes spina Singh (1931). *Mem. Dept. Agric. India, Ent. Ser.* **12(1)**: 27.
Pealius spina (Singh): David and Subramaniam, 1976. *Rec. Zool. Sur. India*, **70**: 209–210.

Specimens examined

3 pupal cases on slides, on *Cassia* sp., Androth (Lakshadweep), 28.iv.1997, Coll: K. Regu.

Hosts

Ficus religiosa L. (Singh, 1931), *Cassia* sp. (new host record).

Distribution

India: Uttar Pradesh, Tamil Nadu (David and Subramaniam, 1976), Lakshadweep (new distribution record).

Ramani (2000) reported the occurrence of *A. dispersus* from Lakshadweep, the first report of an aleyrodid from this Union Territory. In the present study, it was found breeding on two hosts. The record of remaining 11 species *viz.*, *Aleurocanthus* sp., *A. complex*, *A. marlatti*, *B. grossa*, *B. tabaci*, *D. citri*, *D. decempuncta*, *I. loas*, *K. vulgaris*, *M. homonoiae* and *P. spina* forms the first report from Lakshadweep. Among these *B. tabaci* was found breeding on four host plants followed by *D. decempuncta* on three hosts and *A. dispersus* on two hosts. The highly polyphagous nature of whiteflies has been reported by earlier workers (Jesudasan and David, 1991; Srinivasa, 2000). Among the plant species *Thespesia populnea* was found harbouring four species of whiteflies *viz.*, *A. complex*, *A. dispersus*, *A. marlatti* and *B. tabaci*. Considering the small but unexplored area of Lakshadweep, the present contribution emphasises the need for further extensive and intensive study of aleyrodid fauna of Lakshadweep especially related to the ecological and geographical distribution pattern of various genera.

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Changing transporter activity in midgut epithelia of the silkworm after *Bacillus thuringiensis* infection

A. Mahadeva*, M. V. V. Subramanyam and M. P. Shree

Department of Studies in Sericulture, Bangalore University, Jnanabharathi Campus, Bangalore 560056, India
Email: amdeva2002@yahoo.co.in

ABSTRACT: Nutrient absorption in the silkworm, *Bombyx mori* is carried out by ion-coupled transporter at the apical membrane whereas the alkali cation is a Na^+-K^+ ATPases dependent transporter across the basolateral membrane of the midgut epithelium. ATPase activity is not sensitive to chemical inhibitor like Oubain and is not dependent on divalent ions (Mg^{2+}) as well. This enzymatic activity is significantly increased in the midgut epithelia when infected with *Bacillus thuringiensis*. The major cation level (K^+) changes in the haemolymph due to this pump.

KEYWORDS: ATPase, *Bacillus thuringiensis*, cation, oubain, silkworm

The silkworm, *Bombyx mori* is a lepidopteran, monophagous insect voraciously feeding on mulberry, *Morus alba* leaves. The ingested food is digested in the midgut lumen. The midgut of an insect is a barrier for parasites to invasion. This barrier involves chemical composition of the midgut fluid, a non specific surface inhibitor and the peritropic membrane (Tinsley, 1975). The absorption of nutrients from lumen to haemolymph depends a many factors, *viz.*, composition of diet, functioning of digestive enzymes and membrane permeability of intestinal epithelium (Treherne, 1958; Harvey, 1982). The food of phytophagous insect rich in potassium moves passively down with electro-chemical gradient into haemolymph (Harvey *et al.*, 1975). Other nutrients are absorbed from intestinal lumen much faster than can be explained. In lepidopteran midgut membrane, voltage (V_m) is not a combination of sodium and potassium diffusion potentials, but it is a component of hydrogen ion pump potential (Wieczorek *et al.*, 1991; Lapier *et al.*, 1994). Earlier studies in silkworm *B. mori*, have confirmed the presence of carrier mediated transport (Sacchi *et al.*, 1981; Subramanyam and Sarangi, 1989). Alkali metal cation is transported across the epithelium which is generally carried by Na^+-K^+ ATPase pump (Skou, 1964) and has been localized in lepidopteran midgut histochemically (Peacock *et al.*,

*Corresponding author

TABLE 1. Ionic concentration in lumen content, midgut tissue and haemolymph of *B. mori* (meq/L)

	Na^+		K^+		Mg^{2+}	
	Healthy	Infected	Healthy	Infected	Healthy	Infected
Gut content	29 ± 2		91 ± 9		30 ± 7	
Midgut tissue	22 ± 3	19 ± 4	72 ± 6	23 ± 5	54 ± 4	58 ± 5
Haemolymph	15 ± 1	14 ± 2	64 ± 8	53 ± 5	104 ± 11	94 ± 8

Mean ± Standard Deviation.

1972). Jungries and Vaughan (1977) expressed doubts on the presence of Na^+/K^+ exchange pump in these tissues. However, the haemolymph is characterized by high concentration of divalent cations (Florkin and Jeuniaux, 1974). Silkworm is prone to several virulent diseases caused by different bacterial species (Yokayama, 1962) and *Bacillus thuringiensis* is one such species resulting flacherie disease (Ishiwata, 1901). Invading pathogen multiplies in the midgut tissue of silkworm and disturbs normal physiology of digestion. An attempt has been made to know the influence of pathogen on transporter activity and response to activators and chemical inhibitors.

The silkworm (NB_4D_2) rearing was carried out by following Krishnaswamy (1978) method. The second day of fifth instar, larvae were inoculated orally with *Bacillus thuringiensis* (300–400 spores/ml). After two days of inoculation the infected midgut of silkworms were dissected in cold condition; 2.5% homogenate was prepared in Tri-Sucrose buffer and treated with activators ($MgCl_2$, di-Sodium salt) and inhibitor (Oubain). The ATPase activity was measured indirectly by using estimation of inorganic phosphate by Fisk-Subbrow method. The protein was estimated according to Lowry *et al.* (1951). The cation-concentration in midgut and haemolymph were measured in flame photometer (Potassium and Sodium) and atomic absorption spectrophotometer (Magnesium). All the data collected were statistically analysed by Student's *t*-test and expressed as Mean ± SD.

The adult larva of silkworm showed a steep gradient for Potassium (K^+) and Sodium (Na^+) between midgut lumen and haemolymph but also of a relevant Magnesium (Mg^{2+}) ions with higher concentration in the haemolymph (Table 1). Bacterial infection brought changes in the ionic concentration; a decrease K^+ in midgut cells, but slight increase in Mg^{2+}. Na^+ concentration in midgut and haemolymph was not altered by bacterial infection. However, the infected larvae showed a marginal decrease of K^+ and Mg^{2+} in haemolymph. Of all the major cation studied, K^+ ion concentration was altered significantly over other ions on infection.

The midgut ATPase actively revealed a significant increase on bacterial inoculation (Table 2). The enzyme activity was not altered with divalent cation but, it was being stimulated by monovalent ions significantly. Oubain at 10^{-3} M abolished the enzyme activity even in the presence of Na^+ and K^+ when silkworms were inoculated with *B. thuringiensis* (Table 2). The protein content in midgut epithelium was reduced from 0.327 ± 0.04 mg/g tissue to 0.094 ± 0.03 mg/g tissue on bacterial infection.

TABLE 2. ATPase activity in midgut epithelia of *B. mori* infected with *Bacillus thuringiensis*

Reaction media mM/L	ATPase activity in n mole Pi liberate/min/mg protein	
	Healthy	infected
Without activators/inhibitors	1.86	5.34
4 Mg^{2+}	1.07 ± 0.09	5.10 ± 0.29
100 Na^+, 20 K^+	4.07 ± 0.49	15.74 ± 3.17*
100 Na^+, 20 K^+, 10^{-3} M Oubain	1.59 ± 0.14	5.53 ± 0.74

*Significant at 1% level.

Transport of alkali metal cations into and across epithelia has been studied in several tissue (Skou, 1964; Dunham and Hoffman, 1971; Takada and Hasagawa, 1975). In Arthopoda, Oubain sensitive Na^+-K^+ ATPase have been implicated in epithelia Na^+ transport in insects (Baker and Norris, 1971; Rivera, 1975). In lepidopteran insect, the major monovalent alkali cation present in diet and haemolymph is potassium (Jungries *et al.*, 1973; Florkin and Jeuniaux, 1974), whereas sodium is considered as a minor component (Harvey *et al.*, 1975). In such situations, the monovalent K^+ transport across epithelium occurs in the absence of Na^+ counter transport (Harvey and Zerhan, 1972). It is also of worth noting that the active K^+ transport in lepidopteran midgut violates many of the dogma of vertebrate transport physiology (Harvey and Nedergaard, 1964). It is highly probable that it is an electrogenic uniporter (Harvey *et al.*, 1968) though Na^+-K^+ pump is said to be universal. An exception to this rule appears in the midgut epithelia cells of many plant eating lepidopteran insects, with a uncertainty over its presence.

In the present work, the cation pump in the midgut cells was found to be active. This Na^+-K^+ ATPase is very low in silkworm (1.86 n mole). The results showed that it is not Oubain sensitive and not dependent on Mg^{2+}. Similar results were reported by Peacock (1976). Absence of Oubain binding and Na^+-K^+ ATPase in larval adult midgut in three lepidopteran larvae was also reported by Jungries and Vaughan (1977). The presence of very low Na^+ concentration and very high K^+ concentration has been reported in the midgut, haemolymph and in lumen content of *B. mori* and *Philosomia cynthia* (Giordana and Sacchi, 1978). The higher K^+ concentration in all the tissues observed, has been correlated with the higher K^+ concentration in diet (Giordana and Sacchi, 1978; Harvey and Nedergaard, 1964). The present experimental results were agree with earlier workers in having K^+ as a dominant cation in all the tissues considered. The ionic concentration was disequalibrated, when the larvae inoculated with *B. thuringiensis*. The major change observed in K^+ when compared to Na^+ and Mg^{2+}; significant change may be due to change in enzymatic activity of Na^+-K^+ ATPase. This conclusion seems to be valid, as there was significant increase in the enzyme activity in infected silkworms.

The Na^+-K^+ pump is present on the basal membrane which is Oubain insensitive and Mg^{2+} independent. To maintain a gradient of K^+ from lumen to haemolymph,

perhaps there is a K^+/H^+ pump operating at the apical membrane and an electrogenic uniporter, on the basolateral membrane. Apart from Na^+-K^+ ATPase pump, there could be presence of K^+ channel.

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Notes on larval morphology and duration of development of *Aprostocetus hagenowii* (Ratz.) (Hymenoptera: Eulophidae)

Mahabub Hasan and Mahtab Ali*

Department of Zoology, Univeristy of Rajshahi, Rajshahi 6205, Bangladesh

ABSTRACT: *Aprostocetus hagenowii* (Ratz.) an oothecal parasitoid of the cockroach, *Periplaneta americana* deposits eggs on freshly laid ootheca. Determining duration of different stages of development of hymenopteran endoparasites is difficult. In the present study an attempt was made to determine larval duration of the parasitoid on the basis of mandibular measurements. Hatching period and subsequent duration of the larval and pupal instars have been investigated *in vitro*.

KEYWORDS: Larval duration, larval morphology, *Aprostoctus hagenowii*

Although cockroaches have a complement of natural enemies, only a few studies have evaluated these agents for managing cockroach populations (Roth and Willis, 1954b, 1960), Coler *et al.*, 1984; Slater, 1984. *Aprostocetus hagenowii* (Ratzeburg) is a small (1.5 to 2.0 mm) eulophid wasp parasitizing the cockroach egg capsules. The biology and mass rearing of *A. hagenowii*, a dominant parasitoid of *Periplaneta americana* were described by Hagenbuch *et al.* (1988) Cameron (1955); Edmunds (1955) have described the immature stages of *A. hagenowii*, but did not mention the number of larval instars and their duration. An attempt was made here to study the larval duration and larval morphology of *A. hagenowii*.

A pair of newly emerged male and female *A. hagenowii* was placed in a petri dish (8.5 cm diameter) for mating. In this way 100-mated females were obtained. A single mated female parasitoid was released in a petridish containing 14 days old unparasitized oothecae for oviposition. In this way 100, parasitized oothecae were obtained for morphological observations of the immature stages. All female parasitoids were removed from the petridish after allowing 24 h for oviposition. The parasitized oothecae from different petridishes were then placed in an incubator of 20 °C and a relative humidity 78%. At 24h interval, the parasitized oothecae were removed from the incubator one by one and were dissected in 2% saline solution and

*Corresponding author

examined for the presence of parasitoid eggs. The procedure was continued until the appearance of the first instar larvae and subsequently, the other instar larvae, pupae and finally the adults. Data were obtained from at leat 3 sets of observations. The parasitoid eggs floated freely in the oily globules of saline solution, to which aceto-carmine solution was added for staining the parasitoid eggs. The larvae were dissected in glycerine contained in a watch glass. In order to study the tracheal system and body segmentation, the freshly emerged larvae were used (Clausen, 1940; Corbett and Rotherm, 1965). The larvae were washed in a few drops of 10% saline solution in a cavity slide before observation. Hinton's fluid was used to make the larvae transparent for studying the tracheal system (spiracles). For detailed observations, the parasitoid eggs, larvae, pre-pupae, pupae and the preparation of their permanent slides were made. For this they were preserved in lacto phenol solution for 8–10 h and then transferred to 70% alcohol. A gentle teasing of the cuticle above the aforesaid structures with a needle made them more distinct. The larvae and pupae were placed laterally on the slides while the mouthparts of different aged larvae were dissected out , placed dorsally and measurements were taken. Each measurement of specific character was taken from an average of 20 individuals ($N = 20$), with the help of an ocular and a stage micrometer.

In order to determine the larval duration, large number of freshly emerged oothecae were kept in a rearing cage in the laboratory. Then 42 vials were prepared each one containing four freshly laid oothecae. A pair of adult male and female parasitoid was introduced in each vial for oviposition; the mouth of each of the vials was covered with a piece of muslin covered by rubber band. After 24 h all parasitoids were removed from all the vials. In every 24 h each of the four oothecae from a particular vial were dissected for eggs and other immature stages of the parasitoid The immature stages from the dissected oothecae were counted and preserved in 70% alcohol in separate vials. The observations continued for a period of 42 days. The stained larvae in alcoholic eosin were dehydrated with 90 and 100% alcohol and after that glycerin was added. The mandibles were dissected out, were mounted in glycerin and measured with the help of an ocular and a stage micrometer.

In the first instar larva, the head bears a pair of small, chitinized mandibles. The freshly emerged larva is transparent except in the middle part that appears rather opaque and milky, consisting the head and 13 body segments. The thorax forms the widest part of the body while the rest gradually tapers towards the end. The 4th segment is the largest. It is more or less rounded in outline. The body measurements are given in (Table 1). The base of each mandible is rounded whereas the tip is pointed. The mandibles have an average length of 0.29 mm. The second instar larva is similar to first instar except for the larger size and clear segmentation of the body. The mandibles are heavily sclerotized and slightly curved interiorly with a prominent tooth. A pair of notch is present on either side of the posterior part of each mandible. The data on mandible and body measurements are given in (Table 1) . The second instar takes average 5 days to molt to third instar larva. The third instar larva is larger in size with a prominent dark brown mass in the gut, but is similar in color and appearance like

TABLE 1. The length and width of immature stages of *Aprostocetus hagenowii*

Stage	Length ± SE in mm ($N = 20$)	Width ± SE in mm ($N = 20$)
1st instar	2.32 ± 0.03	0.56 ± 0.015
2nd instar	3.21 ± 0.03	3.21 ± 0.03
3rd instar	4.07 ± 0.25	0.77 ± 0.79

TABLE 2. Duration of the immature stages of *Aprostocetus hagenowii*

Stage	Mean duration ± SE in days ($N = 20$)	Mandible size ± SE in mm ($N = 20$)
Egg	2.24 ± 0.04	—
1st instar	4.17 ± 0.29	0.29 ± 0.004
2nd instar	4.80 ± 0.29	0.36 ± 0.03
3rd instar	4.075 ± 0.16	0.48 ± 0.10
Prepupa	1.4 ± 0.11	
Pupa	1.6 ± 0.27	

earlier instars. The average length of the mandibles is 0.48mm (Table 2). The third instar takes an average of 4 days to molt to pupae .

The third instar larva of *A. hagenowii* possess similar features like other eulophid, eupelmid and evaniid parasitoids as reported by several other authors in *Chrysonotomyia* sp. (Mazanec, 1990) *Pediobius imbrues* (Ghosh and Abdurahiman, 1988) *Tetrastichus* sp (Bueno and Fraga, 1988) and *Nesolynx* sp (Bueno *et al.*, 1987). The data gathered in this study indicate clearly that there are three larval stages for *A. hagenowii* The size of the mandibles of the larvae range from 0.29 to 0.48 mm (Table 2). The data obtained through measuring the length of mandibles indicate that 1st and 2nd instar larve take five days to moult to 2nd and third instar larvae respectively and 3rd instar takes four days to moult to pupal stage. The duration of the first instar larva of *A. hagenowii* corresponds to the results presented by Cameron (1955); Narasimham and Sankaran (1982). Islam (1993) and Islam (1995) noted that second instar larvae of *A. caladrae* and *D. basalis* also possessed 13 bodysegments 9 pairs of spiracles, a pair of mandibles and a ring like sclerite which provide apodemes for mandibular articulation. The gut attains dark purple colour. Coats (1976) found that second instar larvae of *M. zaraptor* resembled those of the first instar excepting the larger size of the former. Third instar larvae have sclerotized-paired mandibles and nine pairs of spiracles which is corresponding to the observations by Coats (1976).

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